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# GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS  
BEARING ON HEREDITY AND VARIATION

VOLUME 23 - 1938

WITH SEVEN ~~PLATES~~ PLATES AND ONE HUNDRED AND EIGHTY-ONE TEXT FIGURES

PUBLISHED BY THE  
BROOKLYN BOTANIC GARDEN  
BROOKLYN, NEW YORK  
U.S.A.

# GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS  
BEARING ON HEREDITY AND VARIATION

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Composed, Printed and Bound by  
*The Collegiate Press*  
GEORGE BANTA PUBLISHING COMPANY  
Menasha, Wisconsin

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## CORRIGENDA

VOLUMES 22, 1937 AND 23, 1938

### Volume 22

Page 580, table 3

In "N<sup>1</sup>Xsn bu-3,963 ♀" 250 should read 150

In "sc buXse-1,005 ♂" 110 should read 210

### Volume 23

Page 5, lines 26 and 27 should read: "There are five comparisons provided by subtracting the time for  $p$  from that for  $P$ ."

Page 6, line 7 should read: "Subtracting  $s$  from  $S$  the following differences are taken from Table 3."

Page 8, lines 9-10 should read: "A characteristic of the data that indicates the differential influences of the  $Ss$  pair is that no  $ss$  strain germinated . . . ."

Page 28, footnote\* "Mendel" in place of "Meudel."

Page 59, 13th line from bottom, "sequencies" should be "sequences."

Page 84, addition to last paragraph of section on Material and Methods: "except figure 9, which was reduced to about one-fourth."

Page 201, 2nd line in last paragraph, instead of: "Type A ( $S_1S_3$ )" read "type A ( $S_1S_2$ )."

On page 433, near the end of Table 4 under "Females" "het. B," for "or XXX<sup>pd</sup>," read "or XY<sup>s</sup> or L XX<sup>pd</sup>"; under "y het. B" for "XX" read "XY<sup>s</sup> or L X".

On page 438, last line, for "(c)" read "(b)."

On page 462, on line with "XXX<sup>pd</sup>-eggs" for "12" read "11."

## THEODOR BOVERI

“Die Naturforschung hat ihre dauernden Priester”. JOH. MÜLLER

(*Zur vergleichenden Physiologie des Gesichtssinnes*, 1825)

DER Name THEODOR BOVERI ist mit so vielen Grundtatsachen und theoretischen Vorstellungen verknüpft, auf denen die heutige Vererbungslehre und Entwicklungsphysiologie ruhen, dass er jedem Biologen bekannt ist. Aber gerade weil uns heute so vieles, was BOVERI erarbeitet hat, fast selbstverständlich erscheint, sind wir schon in Gefahr, die Eigenart und den besonderen Wert seiner Arbeitsleistung zu vergessen.

Der äussere Ablauf seines reichen Lebens ist mit wenigen Daten zu umreissen: Er wurde am 12. Oktober 1862 in Bamberg geboren, studierte in München Naturwissenschaften, vor allem als Schüler R. HERTWIGS, und promovierte 1885 zum Dr. phil. 1887 wurde er Privatdozent. 1893 erhielt er den Lehrstuhl der Zoologie und vergleichenden Anatomie in Würzburg. Dort lehrte und forschte er bis zu seinem Tode am 15. Oktober 1915. Eine wertvolle Arbeitsstätte, an der viele seiner Untersuchungen entstanden, war ihm die Zoologische Station in Neapel. In seinem wundervollen Nachruf auf ANTON DOHRN hat er ihr und ihrem Gründer ein Denkmal gesetzt. Seine Erfolge als Forscher verdankte BOVERI dem glücklichen Geschick, dass sich in ihm in ganz ungewöhnlichem Mass Eigenschaften vereinigten, von denen jede einzelne in starker Ausbildung nicht eben häufig ist: die Gabe genauer, unvoreingenommener Beobachtung, reiche Phantasie, die ihn immer neue Fragestellungen und Lösungsmöglichkeiten sehen liess, scharfer, kritischer Verstand und tiefer, sachlicher Ernst. Ohne je “auf die allernächste Hypothesenatmosphäre zu verzichten; ohne die jeder Tatsachenkörper tot bleiben muss,” betonte er streng: “Solange nicht jeder, der zu denken vermag, gezwungen werden kann, die Sicherheit eines Ergebnisses anzuerkennen, solange steht dasselbe, mag die Bedeutung des Problems eine noch so hohe sein, an wissenschaftlichem Rang auf einer untergeordneten Stufe” (1904).

Der Ausgangspunkt für eine Untersuchung war für BOVERI stets eine ganz bestimmte Fragestellung; hiernach wählte er Objekt und Methode aus. Da er “exakte Ergebnisse in dem Sinne, in dem man in Physik und Chemie von Exaktheit spricht,” suchte, konnte seine wesentlichste Methode nur das Experiment sein. Er hat verschiedene Versuchsverfahren sinnreich angewandt; ganz besonders aber liebte er die Ausnützung von Anomalien, die ihm die Natur darbot: “Das Wesentliche des Experiments liegt nur darin, dass man sicher weiss, dass gewisse, sonst stets vorhandene Umstände in einem gegebenen Fall in bestimmter Weise abgeändert worden sind. Wer sie abändert, ob der Beobachter oder die Natur selbst,

ist ganz gleichgültig. Ja, der Forscher am Lebenden wird sich ganz besonders angelegen sein lassen, Abweichungen vom Normalen aufzufinden, bei denen er selbst mit seinen rohen Mitteln gar nicht eingegriffen hat und wo er doch die Art des Veränderten völlig zu durchschauen vermag" (1907).

Drei Tatsachengebiete der Vererbungsforschung hat BOVERI grundlegend gefördert: Das Verhalten der kontinuierlichen Zellbestandteile in Zellteilung, Keimzellenreifung und Befruchtung, die Lokalisation der übertragenen Erbanlagen und das Verhältnis von Kern und Plasma im Entwicklungsgeschehen. Alle drei Gebiete hat er schon in den 80<sup>er</sup> Jahren, noch nicht 30-jährig, betreten. Auf allen ist er bis zu seinem Tode vorangeschritten in steter Ausweitung des Blickfeldes und Vertiefung der Fragestellung. Wir müssen versuchen, auf engem Raum einige Durchblicke durch sein reiches Werk zu gewinnen. Ein Verzeichnis der Schriften BOVERIS mit Ausnahme der letzten (1918) findet sich in dem Nachruf von SPEMANN: Arch. f. Entwickl. mechan. Bd. 42, 1916.

Die erste cytologische Arbeit (1886) handelt "über die Bedeutung der Richtungskörper." Er gab die erste genaue Darstellung des Reifungsvorgangs an *Ascaris megalocephala*, verglich die Eireifung mit der Samenreifung und fasste die Richtungskörper als Abortiveier auf. Auf weitere eigene Untersuchungen an *Ascaris* und die Verknüpfung vorangegangener, unter sich unvermittelt dastehender Entdeckungen von RABL, VAN BENEDEN, O. und R. HERTWIG, FOL u.a. baute BOVERI eine *Theorie der Kernkonstitution, Zellteilung und Befruchtung* auf. Auf Grund der von RABL (1885) gefundenen Tatsache, dass in den Kernen der Salamanderepidermis "bei der Vorbereitung zur Teilung die gleiche Anzahl von Fäden in annähernd der gleichen Lagerung wieder erscheint, die im Beginn der Kernrekonstruktion so charakteristisch hervortritt," betrachtete BOVERI "die chromatischen Elemente als Individuen, elementarste Organismen, die in der Zelle ihre selbständige Existenz führen" (1887). Ferner wird dargelegt, dass "das *Zentrosoma*, das bisher nur als Polkörperchen der Spindel bekannt war, ein selbständiges, dauerndes Zellorgan darstellt, das sich, gerade wie die chromatischen Elemente durch Teilung auf die Tochterzellen vererbt. Es ist das eigentliche Teilungsorgan der Zelle." Die Trennung der Tochterchromosomen und die Zellteilung sind Funktionen der Zentrosomen (1887). Im *Befruchtungsproblem* scheidet BOVERI von vornherein scharf zwischen der "Kombination der Qualitäten zweier Individuen," die er im Einklang mit HERTWIG, WEISMANN u.a. "in höchstem Grade wahrscheinlich" in der "Vereinigung der väterlichen und mütterlichen Kernsubstanz" sieht, und der Entwicklungserregung, dem Anstoss zur Teilung des Eis. Nach BOVERIS Auffassung findet im reifen Ei eine Rückbildung des *Zentrosomas* statt; das Spermatozoon bringt ein Zentro-

soma in das Ei hinein und stellt damit die Teilungsfähigkeit wieder her.— Die Diskussionsbemerkungen von KUPFFER, R. HERTWIG und BONNET zu den ersten Mitteilungen BOVERI in der Gesellschaft für Morphologie und Physiologie in München (1886, 1887) zeigen, wie neu alle diese Anschauungen damals noch waren.

Auf die weitere Aufklärung der Zellteilungsphysiologie und der Kernkonstitution hat BOVERI weiterhin noch eine Fülle von Mühe und Scharfsinn verwandt. Die "*Theorie der Chromosomenindividualität*" hat er in grossartiger Beweisführung sichergestellt. Seine Zusammenfassung "Ergebnisse über die Konstitution der chromatischen Substanz des Zellkerns" (1904) ist ein klassisches Buch unserer Wissenschaft.

Die Frage ob tatsächlich die *Erbanlagen* "ausschliesslich im Kern enthalten seien," liess sich nur auf dem einzigen Wege lösen, "dass man von zwei verschiedenartigen Zellen von der einen das Protoplasma, von der andern den Kern nimmt und diese Teile zu einer neuen Zelle vereinigt."

Eine Möglichkeit zur Lösung des Problems schien die Ausnützung der Entdeckung von O. und R. HERTWIG zu bieten, dass kernlose Eifragmente sich befruchten lassen. Für den scheinbaren Erfolg eines solchen "*Meroгонieversuchs*," in dem BOVERI Eibruchstücke von *Sphaerechinus* mit Samen von *Parechinus* befruchtete und einzelne Larven von rein väterlichem Skeletttypus bekam (1889), hat BOVERI allerdings später (1918) eine Fehlerquelle aufgezeigt. Aber BOVERI hat weiterhin zwei andere höchst elegante Beweise dafür geliefert, dass die Erbanlagen, welche die Ausbildung der Larvencharaktere der Seeigel bestimmen, im Kern liegen und auf die verschiedenen Chromosomen verteilt sind: durch den *Dispermieversuch* (1892) und den *Rieseneierversuch* (1914). Nach Befruchtung mit 2 Spermien entstehen gewöhnlich 4 Spindelpole, und das Ei wird simultan in 4 Blastomeren geteilt. Dabei werden die Tochterchromosomen der 3 vorhandenen Chromosomensätze rein zufallsmässig auf die 4 Pole und die 4 Blastomeren verteilt. Zieht man die 4 ersten Blastomeren eines normalen Keimes getrennt auf, so wird aus jeder eine normal gebaute, entsprechend verkleinerte Larve. Die vier simultan aus einem dispermen Ei entstandenen Blastomeren entwickeln sich getrennt aufgezogen in der ganz überwiegenden Mehrzahl abnorm und unter sich verschieden. In ihrem Plasmabestand gleichen sie einander und den Blastomeren des normalen Vierzellenstadiums; die Störung und die Verschiedenartigkeit ihrer Entwicklung kann also nur auf einer Abänderung der Chromosomenkombination beruhen, womit bewiesen ist, dass die Chromosomen verschiedenwertig sind. Der "experimentelle Beweis, dass die Übertragung der spezifischen mütterlichen Eigenschaften nicht durch das Eiplasma sondern durch den Eikern geschieht," wurde von BOVERI (1914), gleichzeitig mit C. HERBST, durch die Bastardierung von Rieseneiern erbracht:

Das Riesenei hat doppelte Chromosomenanzahl und doppelte Plasma-grösse; die Vererbungsrichtung von Artbastarden wird nach der Mutter-seite verschoben. Die Mutterähnlichkeit kann nicht auf der Plasmaver-mehrung beruhen; denn gleichzeitig wurden Eifragmente mit Spermien der gleichen Männchen befruchtet, und es zeigte sich, dass die mütterliche Tendenz mit der Verminderung des Eiplasmas nicht abnimmt. Damit ist bewiesen, dass die Vererbungssubstanz im Kern liegt und quantitativ wirkt.

Die Verschiedenwertigkeit der Chromosomen und die genaue Paral-lelität der Chromosomenverteilung in der Keimzellenreifung und Befruch-tung mit der Verteilung der Erbfaktoren nach den Mendelschen Gesetzen führte BOVERI, gleichzeitig mit SUTTON, zu der *Theorie der Lokalisation der Mendelschen Erbfaktoren in den Chromosomen* (1903).

Die Erfahrungen über die zur Keimesentartung führende Wirkung von Abänderungen der normalen Chromosomenkombination regten BOVERI zu einem theoretischen Aufsatz "zur Frage der *Entstehung maligner Tumoren*" (1914) an. Hier entwickelt BOVERI die Hypothese, dass die Krebszelle sich von der normalen unterscheide "durch einen gewissen abnormen Chromatinbestand, gleichgültig, wie er entsteht." Grundgedanken dieser ideenreichen Schrift, in der ein grosses Tatsachenmaterial verarbeitet ist, leben in modernen Krebs-hypothesen wieder auf.

Auch mit dem Problem der *Geschlechtsbestimmung* hat sich BOVERI beschäftigt. Mit einer Reihe von Schülern untersuchte er das Vorkommen von Geschlechtschromosomen bei Nematoden und bemühte sich um die Aufklärung der Chromosomenverhältnisse bei einer hermaphroditischen Art (1911). Ein besonders reizvolles Problem boten ihm die "*Eugsterschen Zwitterbienen*." Er hatte bei Seeigeln (1888) beobachtet, dass ausnahms-weise der Spermakern vom Eikern getrennt bleiben und später mit dem Kern der einen Blastomere verschmelzen kann. Er wies schon damals darauf hin, "dass, wenn in einem befruchteten Bienen-ei die gleiche Ab-normität auftreten würde, höchstwahrscheinlich ein gynandromorphes Individuum die Folge sein würde." Morgan stellte (1905, 1909) gegenüber der Annahme BOVERIS, dass die männlichen Teile der Zwitterbienen reine Eikerne enthalten, die Hypothese auf, dass sie gerade umgekehrt nur mit Spermaabkömmlingen ausgestattet seien, wofür sich auch Modellfälle von disperm befruchteten Seeigeleiern, bei denen ein Eikern selbständig ge-blieben war, anführen liessen. Eine Entscheidung zwischen beiden Möglich-keiten liess sich erbringen, da die Eltern der von BOVERI (1915) eingehend untersuchten Eugsterschen Zwitterbienen verschiedenen Rassen ange-hörten: Die männlichen Teile entsprachen rein der Rasse der italienischen Mutter (*Apis mellifica ligustica*), während die weibliche Seite eine Mi-schung des *ligustica*- und *mellifica*-Typus aufwies; BOVERIS Hypothese wurde also bestätigt.

Auf das *Determinationsproblem* wurde BOVERI schon 1887 durch die Entdeckung der Chromatindiminution bei *Ascaris megalocephala* geführt. Später konnte BOVERI durch Verlagerung von Inhaltsbestandteilen durch Zentrifugieren, durch Abtöten bestimmter Zellen durch ultraviolettes Licht und besonders wieder durch Auswertung disperm befruchteter Eier tief in das Determinationsgeschehen im Ascaridenkeim eindringen, vor allem die Frage nach der *Verursachung der Chromatindiminution* beantworten. Das Ascaris-Ei besitzt vor der Furchung einen ausgesprochen heteropolen Bau; wir können eine animale und eine vegetative Hälfte an ihm unterscheiden. Bei der normalen Entwicklung kommt bei der ersten Teilung ein Spindelpol in die animale, der andere in die vegetative Hälfte zu liegen. In der animalen Zelle tritt Diminution ein, in der vegetativen nicht. Im disperm befruchteten Ei können sich die Spindelpole verschieden einstellen und entweder 2 animale und 2 vegetative oder 1 animale und 3 vegetative oder 3 animale und 1 vegetative Zelle gebildet werden; und jedesmal werden im animalen Bereich die Kerne diminuiert, im vegetativen bleiben die Urchromosomen erhalten. Das Verhalten der Chromosomen wird also durch ihre Umgebung bestimmt. "So scheint der Fall von Ascaris ein einfaches Paradigma dafür darzustellen, wie die Wechselwirkung von Protoplasma und Kern in der Ontogenese zu denken ist, und auf welche Weise aus der äusserst geringen Ungleichartigkeit des Ei-*protoplasmas*, durch Auslösungswirkungen auf den Kern und Rückwirkungen vom Kern auf das Protoplasma, die schliesslich so gewaltigen Verschiedenheiten der entstehenden Zellen hervorgehen können" (1910).

In einer anderen Richtung trieb BOVERI das Kern-Plasma-Problem in seiner letzten Arbeit vor, die MARCELLA BOVERI nach dem Tode ihres Mannes herausgegeben hat (1918). Das *Merogonieproblem* hatte BOVERI nie Ruhe gelassen. Eine Wiederholung der alten Versuche führte auf Fehlerquellen: Durch das Schütteln kann der Eikern als Ganzes oder in Form von Partialkernen unsichtbar werden; so ist es möglich, dass ein scheinbar kernloses Eistück doch einen Kern oder ein Kernstück enthält. Die Feststellung der Kerngrössen in zahlreichen neuen Zuchten aus zerschüttelten Sphaerechinus-Eiern, die mit Paracentrotus- oder Parechinus-Sperma befruchtet waren, zeigte, dass die eikernlosen Fragmente sich zunächst ebensogut entwickeln wie die eikernhaltigen. Nach Vollendung des Blastulastadiums stellen jene aber stets die Weiterentwicklung ein. Hierdurch kam BOVERI zu der Überzeugung, dass auch in seinen Versuchen von 1889 die wenigen vermeintlich merogonischen, rein väterlich aussehenden Plutei doch einen Teil des Sphaerechinus-Kerns enthalten hatten und damit jene Ergebnisse aus der Diskussion ausscheiden müssen. Aber in anderer Richtung waren durch diese neuen ausgedehnten Merogonieversuche sehr wichtige Ergebnisse über die *Rolle von Ei-Plasma und Kern*

*in der Embryonalentwicklung* gewonnen: "Gewisse allgemeine Formverhältnisse des entstehenden Individuums sind ohne Zweifel in der Anordnung des Eiplasmas vorgezeichnet. Hier spielen offenbar die Gene noch gar keine Rolle, wenn nicht etwa in der Weise, dass sie bei der Bildung des Eies (der Oocyte) dessen Plasmabau beeinflussen können." Hiermit "steht in bester Übereinstimmung, dass das Ei seine erste Entwicklung mit einem, wie man fast sagen kann, beliebigen Chromatinbestand auszuführen vermag." Vom Beginn der Gastrulation an ist "die Weiterentwicklung von der Anwesenheit eines zum Eiplasma richtig abgestimmten Chromosomenkomplexes abhängig." Ermöglicht der Eikern oder ein Stück von ihm die weitere Entwicklung, so kann der väterliche Kern auf dieses Entwicklungsgeschehen, das er durchaus nicht in Gang zu setzen vermöchte, formativ mit solcher Kraft einzuwirken, dass in dem entstehenden Pluteus die Entfaltung der mütterlichen Anlagen völlig unterdrückt wird. So ergab sich ein neuer, weiter Ausblick: "Bezeichnet man als Vererbung die Gesamtheit der inneren Bedingungen, die zur Entfaltung der Eigenschaften des neuen Individuums gehören, so kommt hierbei dem Protoplasma eine viel spezialisiertere Bedeutung zu, als man bisher vielfach anzunehmen geneigt war," und "die Rolle des Kerns in der Entwicklung fällt nicht zusammen mit der Übertragung der elterlichen Qualitäten," d.h. der Bestimmung einer Summe einzelner sichtbarer Merkmale. Wir sehen, welche Probleme BOVERI jetzt am meisten bewegten: "Über die Frage, wie die in der Zygote gegebene Konstitution zu jenem Erbefekt hinführt, mit dem der Vererbungsforscher arbeitet, wissen wir trotz aller exakten Vererbungslehre so gut wie nichts; . . . ja man wird vielleicht, wenn man an die jetzt bekannten Angriffsmöglichkeiten denkt, an der Lösbarkeit einer solchen Aufgabe überhaupt verzweifeln" (1918).

Immer wieder, besonders beim Lesen der letzten Arbeit BOVERIS, welche die ganze Meisterschaft des Forschers so eindrucksvoll zeigt, empfinden wir es tief schmerzlich, dass BOVERI die Weiterentwicklung der Genlokalisierungstheorie und die sich anbahnende Verknüpfung von Genetik und Entwicklungsphysiologie nicht miterlebte. Wie hätten ihn diese Vertiefung der Erkenntnis und diese Blosslegung neuer Problemschichten beglückt! Wie hätte sein weiteres Schaffen uns bereichert!—"Die Naturforschung hat ihre dauernden Priester,"—ihre Gemeinschaft hoch zu halten ermahnt uns immer die geistige Gestalt BOVERIS.

ALFRED KÜHN (Berlin-Dahlem).

*The portrait of Theodor Boveri is from a photograph kindly loaned by Mrs. Theodor Boveri.*

# THE RELATION OF THE FIRST CHROMOSOME PAIR TO DATE OF FRUIT RIPENING IN THE TOMATO (LYCOPERSICUM ESCULENTUM)<sup>1</sup>

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Received Aug. 17, 1937

THE linear order of four genes in chromosome I of the tomato has been well established, principally from data obtained by LINDSTROM (1932) and McARTHUR (1934). LESLEY (1931) has shown one of the genes in this linkage group to be related to resistance to the curly top virus disease, and LINDSTROM (1932) has demonstrated a decided relationship between fruit size and the genes in this pair of chromosomes. YEAGER (1937) has data that demonstrate the presence of a fifth gene in the group and considers this to be a major gene for fruit shape and size.

The four genes that have been located by linkage tests and their arrangement are as follows:

- d* dwarf plant recessive to *D*, standard plant
- p* pubescence on the fruit recessive to *P*, smooth fruit
- o* pear shape fruit recessive to *O*, round fruit
- s* compound inflorescence recessive to *S*, simple inflorescence

McARTHUR (1934) has stated that certain genes affect the rate of fruiting, noting especially that the *Ll* (lutescent) pair in chromosome V retards ripening when recessive.

CURRENCE (1932) found other genes to be associated with time of ripening fruit. Ripening dates of phenotypic alleles resulting from crosses of recessive by dominant of different dates of ripening were compared, and a relationship of the *Dd* genes to season of ripening was found. This report consists of additional material on the effect which the region associated with the four marker genes of the first chromosome has on the time of fruit ripening.

## MATERIAL AND METHODS

Seed of a strain homozygous for *d p o s* was obtained from Dr. E. W. LINDSTROM. A representative plant from this strain was crossed with a plant of the variety known locally as Danish Export, an early ripening type producing fruit a few days earlier than average strains of the well known Earliana variety, and dominant for the four genes of the first chromosome. A plant from a small F<sub>1</sub> population was used as a pistillate parent and pollen from the *d p o s* parent was applied. Plants were then

<sup>1</sup> Paper No. 1555, Scientific Journal Series, Minnesota Agricultural Experiment Station.



grown from this backcross and compared for earliness with plants resulting from selfing both parents and with the  $F_1$  plants.

The seed was started in flats and all seedlings were transplanted in about two weeks into  $3\frac{1}{2}$ -inch clay pots. The plants were grown in these pots for a period of approximately one month and then set into the field. Care was exercised to get them into the field before any flowering began since failure of pollination often interferes with fruit setting early in the season and this would cause inequality in ripening.

The segregates were classified for the four marker genes in the backcross population and all plants of normal appearance were classified for earliness by recording the date at which they developed the first ripe fruit.

#### EXPERIMENTAL DATA

*Map distances.* Inasmuch as several hundred backcross plants have been classified for the four qualitative characters, the data may be used to substantiate further the linkage relationships published by LINDSTROM (1932) and McARTHUR (1934). Table 1 contains the linkage figures ob-

TABLE 1

*Recombination values calculated by combining all available backcross data.*

| COMBINATIONS | NUMBER | DATA BY<br>LINDSTROM | TOTAL | PERCENT |
|--------------|--------|----------------------|-------|---------|
| <i>DPOS</i>  | 377    | 125                  | 502   |         |
| <i>dpos</i>  | 163    | 85                   | 248   | 68.8    |
| <i>DPos</i>  | 19     | 14                   | 33    |         |
| <i>dPOS</i>  | 35     | 20                   | 55    | 8.1     |
| <i>DPOS</i>  | 73     | 16                   | 89    |         |
| <i>dpos</i>  | 97     | 13                   | 110   | 18.3    |
| <i>Dpos</i>  | 13     | 4                    | 17    |         |
| <i>dPOS</i>  | 12     | 4                    | 16    | 3.0     |
| <i>DPOS</i>  | 2      | 6                    | 8     |         |
| <i>dpos</i>  | 4      | 0                    | 4     | 1.1     |
| <i>Dpos</i>  | 4      | 1                    | 5     |         |
| <i>dPOS</i>  | 1      | 1                    | 2     | 0.6     |
| <i>DPOS</i>  | 0      | 0                    | 0     |         |
| <i>dPos</i>  | 1      | 0                    | 1     | 0.1     |
|              | 801    | 289                  | 1090  | 100.0   |

tained when those of the present study are combined with those published by LINDSTROM. Slight changes in the loci of *Oo* and *Ss* are suggested by the combined data. Figure 1 shows the recombination relationships of the four genes.

Since the parents were different in earliness and also differed in the four qualitative characters, the backcross segregates for the qualitative characters show any association that might exist between them and earliness. If any one of the four marker genes is in a section of the chromosome that affects rate of development, the crossovers involving that gene would in general be affected by the time of ripening gene or genes associated with it in the parent plant.

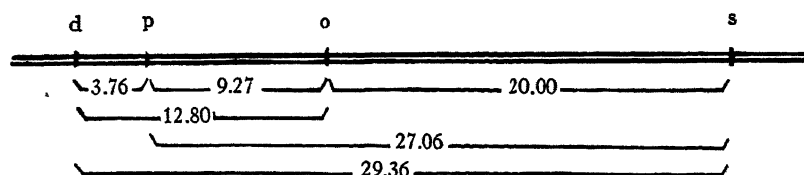


FIGURE 1.—Recombination values of four genes in the first chromosome.

Table 2 gives the summarized data on earliness in parents,  $F_1$  and in the various phenotypes in the backcross population. The standard errors of the means are given for each generation or phenotype. The generalized standard deviation of 4.49 days for the backcross groups was calculated by the analysis of variance method from the sums of squares within

TABLE 2  
Distribution of ripening dates for various phenotypes resulting from a backcross of  $F_1$   
( $DPOS$  early  $\times dpos$  late)  $\times dpos$  late.  
Days from August 1 to ripening of fruit.

| GENERATION | PHENOTYPES | 12 | 16 | 20 | 24  | 28 | 32 | 36 | 40 | 44 | 48 | 52 | 56 | 60 | TOTAL | MEAN            |
|------------|------------|----|----|----|-----|----|----|----|----|----|----|----|----|----|-------|-----------------|
| $P_1$      | $DPOS$     | 2  | 3  | 10 | 9   | 19 | 11 | 11 | 1  |    |    |    |    |    | 66    | $27.4 \pm 0.77$ |
| $P_1$      | $dpos$     |    |    |    |     |    |    | 1  | 8  | 10 | 27 | 2  | 2  | 1  | 51    | $46.4 \pm 0.62$ |
| $F_1$      | $DPOS$     |    |    | 15 | 22  | 26 | 5  | 2  |    |    |    |    |    |    | 70    | $25.6 \pm 0.47$ |
| BC         | $DPOS$     | 6  | 12 | 70 | 123 | 78 | 21 | 7  |    |    |    |    |    |    | 317   | $24.4 \pm 0.25$ |
| BC         | $DPos$     |    | 1  | 3  | 12  | 27 | 13 | 4  | 2  |    |    |    |    |    | 62    | $28.4 \pm 0.57$ |
| BC         | $DPos$     |    |    |    |     | 1  | 1  |    |    |    |    |    |    |    | 2     | $30.0 \pm 3.18$ |
| BC         | $DPos$     |    |    |    | 1   | 1  | 5  | 9  | 1  |    |    |    |    |    | 17    | $29.9 \pm 1.09$ |
| BC         | $Dpos$     |    |    |    |     |    |    |    |    |    |    |    |    |    | 0     | —               |
| BC         | $Dpos$     |    |    |    |     |    |    |    |    |    |    |    |    |    | 0     | —               |
| BC         | $Dpos$     |    |    |    |     | 1  | 2  |    |    |    |    |    |    |    | 3     | $26.7 \pm 2.59$ |
| BC         | $Dpos$     |    |    |    |     | 1  | 6  | 3  | 1  |    | 1  |    |    |    | 12    | $34.7 \pm 1.30$ |
| BC         | $dPOS$     |    |    |    | 1   | 2  | 1  | 5  |    |    |    |    |    |    | 9     | $32.4 \pm 1.50$ |
| BC         | $dPOS$     |    |    |    |     |    |    |    | 1  |    |    |    |    |    | 1     | $36.0 \pm 4.49$ |
| BC         | $dPos$     |    |    |    |     |    |    |    |    |    |    |    |    |    | 0     | —               |
| BC         | $dPos$     |    |    |    |     |    |    | 1  |    |    |    |    |    |    | 1     | $32.0 \pm 4.49$ |
| BC         | $dpos$     |    |    |    | 1   | 1  | 4  | 12 | 8  | 3  | 1  |    |    |    | 30    | $37.1 \pm 0.82$ |
| BC         | $dpos$     |    |    |    |     |    |    | 1  | 2  |    |    |    |    |    | 3     | $38.7 \pm 2.59$ |
| BC         | $dpos$     |    |    |    | 1   | 3  | 20 | 31 | 22 | 9  |    |    |    |    | 86    | $36.5 \pm 0.48$ |
| BC         | $dpos$     |    |    |    |     |    |    | 9  | 39 | 45 | 21 | 10 | 3  |    | 127   | $43.8 \pm 0.40$ |

phenotypes. Only those groups containing nine or more plants were used in the calculations. In order to have the backcross data as complete as possible, the generalized standard deviation is used as the standard error of a single plant, and therefore may be of some value if applied as such to

TABLE 3

Differences in ripening dates for various phenotypes resulting from a backcross of  $F_1$  ( $DPOs$  early  $\times$   $dpos$  late)  $\times$   $dpos$  late. Vertical column subtracted from horizontal.

| NUMBER OF<br>PLANTS | 62                  | 2           | 17          | 3           | 12          | 9           | 1           | 1           | 30          | 3           | 86          | 127         |            |
|---------------------|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------|
|                     | NUMBER<br>OF PLANTS | <i>DPOs</i> | <i>DPoS</i> | <i>DPoS</i> | <i>DpoS</i> | <i>Dpos</i> | <i>dPOS</i> | <i>dPOs</i> | <i>dPos</i> | <i>dPOS</i> | <i>dPoS</i> | <i>dpos</i> |            |
| <i>DPOS</i>         | 317                 | 4.0<br>**   | 5.6         | 5.5<br>**   | 2.3         | 10.3<br>**  | 8.0<br>**   | 11.6<br>*   | 7.6         | 12.7<br>**  | 14.3<br>**  | 12.1<br>**  | 19.4<br>** |
| <i>DPoS</i>         | 62                  |             | 1.6         | 1.5         | 1.7         | 6.3<br>**   | 4.0<br>*    | 7.6         | 3.6         | 8.7<br>**   | 10.3<br>**  | 8.1<br>**   | 15.4<br>** |
| <i>DPoS</i>         | 2                   |             |             | 0.1         | -3.3        | 4.7         | 2.4         | 6.0         | 2.0         | 7.1<br>*    | 8.7<br>*    | 6.5<br>*    | 13.8<br>** |
| <i>DPos</i>         | 17                  |             |             |             | 3.2         | 4.8<br>**   | 2.5         | 6.1         | 2.1         | 7.2<br>**   | 8.8<br>**   | 6.6<br>**   | 13.9<br>** |
| <i>DpoS</i>         | 3                   |             |             |             |             | 8.0<br>**   | 5.7         | 9.3         | 5.3         | 10.4<br>**  | 12.0<br>**  | 9.8<br>**   | 17.1<br>** |
| <i>Dpos</i>         | 12                  |             |             |             |             |             | -2.3        | 1.3         | 2.7         | 2.4         | 4.0         | 1.8         | 9.1<br>**  |
| <i>dPOS</i>         | 9                   |             |             |             |             |             |             | 3.6         | 0.4         | 4.7<br>**   | 6.3<br>*    | 4.1<br>**   | 11.4<br>** |
| <i>dPOs</i>         | 1                   |             |             |             |             |             |             |             | 4.0         | 1.1         | 2.7         | 0.5         | 7.8        |
| <i>dPos</i>         | 1                   |             |             |             |             |             |             |             |             | 5.1         | 6.7         | 4.5         | 11.8<br>** |
| <i>dPoS</i>         | 30                  |             |             |             |             |             |             |             |             |             | 1.6         | -0.6        | 6.7<br>**  |
| <i>dPoS</i>         | 3                   |             |             |             |             |             |             |             |             |             |             | 2.2         | 5.1        |
| <i>dpos</i>         | 86                  |             |             |             |             |             |             |             |             |             |             |             | 7.3<br>**  |

\* Exceeds 5% point.

\*\* Exceeds 1% point.

those groups having populations of fewer than nine plants. Although it is not intended to base conclusions on these small numbers it will be noted that in many cases they gave results that were statistically significant with the above error applied to them. The standard errors of all the backcross means were obtained by dividing the generalized standard deviation by the square root of the number of plants in the particular group, and those for the parents and  $F_1$  were obtained by the formula  $\sqrt{\Sigma fd^2/n}$ .

The difference between the parents was approximately 19 days. The  $F_1$  was uniform and slightly earlier than the early parent. Among the backcross plants the individuals with the four dominant marker genes were the earliest and those with the four recessive genes were the latest of the phenotypes. The phenotypes representing crossovers in the three

regions were intermediate for date of ripening. In general, the earliness of the groups was directly related to the number of dominant marker genes present.

By appropriate comparisons of ripening dates among the groups, the effect which specific regions of the chromosome might have on the rate of development is shown. Certain comparisons also provide information as to the extent of any interacting effects of the different parts of the chromosome. The differences between all phenotypes are given and their statistical significance determined by FISHER'S table of *t* values in table 3. The degrees of freedom "within phenotypes" were used. The comparisons which follow indicate the extent to which the *Dd* region may affect earliness. The number of plants is given in parentheses.

*DPOS* (317)—*dPOS* (9) = -8.0 highly significant

*Dpos* (12)—*dpos* (127) = -9.1 highly significant

*DPOs* (62)—*dPOs* (1) = -7.6

*DpoS* (3)—*dpoS* (86) = -9.8 highly significant

*DPos* (17)—*dPos* (1) = -2.1

Since the differences for the two comparisons with satisfactory numbers are highly significant, there is sufficient evidence that this end of the chromosome has a differential effect of about 8 days on the time of maturity. So far as the data show, the difference is not greatly modified by changes in other chromosomal regions. The difference of 1.1 days between the two differences is not statistically significant.

The second region, which is the one containing the gene *p* for pubescence on the fruit, appears to exert an effect on the rate of development to the fruit ripening stage. There are five comparisons provided by subtracting the time for *P* from that for *p*.

*DPoS* (17)—*DpOs* (12) = -4.8 highly significant

*dPOS* (9)—*dpOS* (30) = -4.7 highly significant

*dPos* (1)—*dpos* (127) = -11.8 highly significant

*DPoS* (2)—*DpOS* (3) = +3.3

*dPOS* (1)—*dpOS* (3) = -2.7

The two comparisons *DPoS*—*DpOs* and *dPOS*—*dpOS* have numbers that are probably large enough to provide accurate information. Based on these two pairs of phenotypes, *P* appears to hasten ripening over *p* by about 5 days. The other comparisons which involve small numbers are of limited value. However they suggest the possibility that the differences between *P* and *p* might vary in different combinations of the other genes.

The *Oo* region offers comparisons that are of interest. None of the pairings for this region of the chromosome show significant differences; and

those with sizeable populations have differences that are very small. It is therefore probable that this part of the chromosome does not contain important factors for early ripening. However, it may contain genes of minor importance since most of the above comparisons show the *O* plants to be earlier than those having *o*.

The fourth region which contains the gene *s* affecting type of inflorescence appears to influence the rate of development. Subtracting *S* from *s*, the following differences are taken from table 3.

$$\begin{aligned} D P O S (317) - D P O s (62) &= -4.0 \text{ highly significant} \\ d p o S (86) - d p o s (127) &= -7.3 \text{ highly significant} \\ D p o S (3) - D p o s (12) &= -8.0 \text{ highly significant} \\ d P O S (9) - d P O s (1) &= -3.6 \end{aligned}$$

The effect of the *Ss* region appears to be distinct and significant. The varying effect of the region in the two different combinations where numbers are large, is of interest since it suggests an interaction of genes for earliness. The difference between the two differences is  $3.3 \pm 0.9$ . The genes associated with *S* may be considered to be more effective in shortening the time to fruit ripening when not in combination with the genes for earliness that are associated with the *D P O* section of the chromosome. It may be that only a part of the *D P O* region is responsible for the interaction. There is some inconclusive evidence that the interaction does not involve *D d*.

From the foregoing results there are at least three genes which affect earliness of fruiting. Their effect is thought to be accumulative, but in one case it is smaller in combination with other genes for earliness. In the cross studied, there are no indications that the *Oo* region has any important bearing on the date of fruit ripening.

This statement is based mostly on the slight differences between *D P O s*—*D P o s*, and *d p O S*—*d p o S*. The numbers in each of these four groups are satisfactory. The mean differences are small, and the distributions are quite similar in both cases. It therefore seems that the genes responsible for the association of *P* and *S* with earliness must be either very near these genes, within the loci, or on the sides away from *O*. Otherwise the *Oo* crossovers should on occasion be affected by the same genes that affect the *Pp* and *Ss* crossovers. Therefore, the outer ends of that part of the chromosome studied seem to be of most importance to earliness. Crossing over between *d* and *p* and between *p* and *o* provide some evidence that there is an effect on earliness, quite close to the *Dd* locus or to the left of it. The *D P o s* group of plants was 13.9 days earlier than the *d p o s* plants whereas the *D p o s* group differed from the *d p o s* by 9.1 days. Consider that the effect connected with *P* has been shown

to be probably either within the locus or to the left of it, and that *D* increases this effect. This increase would be impossible if *D* were not more closely associated with the particular cause than was *P*. Therefore there is a gene or possibly genes for earliness that lie closer to *Dd* than to *Pp*. The small number of crossover plants for the region between *d* and *p* is somewhat insufficient for satisfactorily separating the effects of the two regions. An extensive number of such crossovers would indicate whether or not the associations with *Pp* and *Dd* were independent of the chromosome connecting the two genes. Figure 2 is shown for the purpose of presenting diagrammatically such approximate effects as noted for the different chromosome sections.

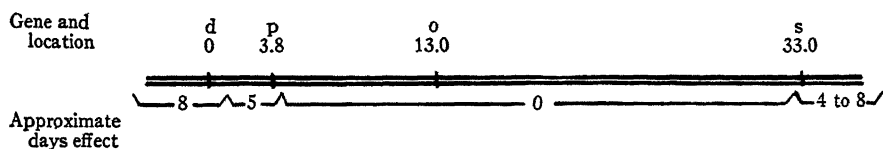


FIGURE 2.—Effects on ripening time of different regions of chromosome I.

That the part of the chromosome studied may affect the rate of the plant's development very early in the life cycle, is shown by the data on germination that are given in table 4. Thirty-five seeds from each of various self pollinated backcross plants were planted in greenhouse flats and records made of the number of days before the seedlings emerged. Grouping and averaging the means of individual lines according to the phenotype of the parent plant provided the results shown in the table. A generalized standard error for the averages of the means was calculated by the analysis of variance method basing the variability on the sum of squares within phenotypes and using only the five groups that contained nine or more lines for the calculations. The standard error divided by the square root of the number of lines in the phenotype gives the standard error of the group of means. Again with these figures, the errors have been applied to the smaller populations with the assumption that they have a certain value in supplementing the information from larger groups.

It is apparent that the phenotype *D P O S* of the earlier parent was significantly faster in germinating than the *d p o s* group of lines. However, individual regions with the exception of *S—s* do not show significant differences. Of the *S—s* comparisons, there are two which have sufficient numbers to provide definite information. *D P O S* was one day faster in germinating than *D P O s*, and *d p o S* germinated 1.7 days sooner than *d p o s*. In both instances, the *t* table shows the differences to be highly significant. The data do not indicate important association between time of germinating and any of the other individual regions, but there are certain comparisons showing significant differences by adding

TABLE 4

Mean averages of days to germinate and percentage germination of lines produced by self pollinating plants of various backcross phenotypes.  $F_1(DPOS \text{ early} \times dpos \text{ late}) \times dpos \text{ late}$ .

| PHENOTYPE OF<br>PARENT PLANT | NUMBER OF<br>LINES | DAYS TO<br>GERMINATE | PERCENTAGE<br>GERMINATION |
|------------------------------|--------------------|----------------------|---------------------------|
| <i>DPOS</i>                  | 25                 | 7.90 ± 0.21          | 92.8 ± 1.8                |
| <i>DPOs</i>                  | 11                 | 8.91 ± 0.31          | 78.2 ± 2.8                |
| <i>DPoS</i>                  | 2                  | 6.75 ± 0.73          | 92.5 ± 6.5                |
| <i>DPos</i>                  | 5                  | 9.10 ± 0.46          | 73.0 ± 4.1                |
| <i>DpOS</i>                  | 1                  | 7.00 ± 1.04          | 95.0 ± 9.2                |
| <i>DpOs</i>                  | 3                  | 7.50 ± 0.60          | 90.0 ± 5.3                |
| <i>Dpos</i>                  | 7                  | 10.21 ± 0.39         | 67.1 ± 3.5                |
| <i>dPOS</i>                  | 6                  | 8.58 ± 0.42          | 93.3 ± 3.8                |
| <i>dPOs</i>                  | 1                  | 10.00 ± 1.04         | 90.0 ± 9.2                |
| <i>dPos</i>                  | 1                  | 10.00 ± 1.04         | 75.0 ± 9.2                |
| <i>dpOS</i>                  | 9                  | 7.94 ± 0.35          | 94.4 ± 3.1                |
| <i>dPOs</i>                  | 3                  | 9.67 ± 0.60          | 68.3 ± 5.3                |
| <i>dpos</i>                  | 9                  | 8.56 ± 0.35          | 90.0 ± 3.1                |
| <i>dpOs</i>                  | 10                 | 10.25 ± 0.33         | 82.0 ± 2.9                |

individual regions; thus *DPOs* vs *DpOs* gives 1.3, significant. Here the *PO* region is compared with *po*, and it is apparent that the effect of these two regions is accumulative in relation to rate of germinating. Other comparisons also show significant differences but small numbers are involved in all of them.

Data on percentages of germination were also analyzed and given in table 4. Here again is a noticeable effect associated with the *Ss* pair of genes, and a somewhat general similarity between these figures and those on time of germinating. A characteristic of the data that indicates the differential influences of the *Ss* pair is that no *Ss* strain germinated in the 100 percent class and none with *Ss* occurred in the extremely low classes, but recessives of other genes were in the 100 percent group and dominants for them occurred in the lower classes. The two *Ss* comparisons, *DPOS*—*DPOs* and *dpos*—*dpOs* have differences of 14.6 and 8.0 respectively. The former exceeds the one percent point and the latter is slightly below the five percent point with odds of 16.5 to one. The difference between the differences is  $6.6 \pm 3.3$ , a *t* value that approximately equals the five percent point.

By analysis of covariance, the figures in table 4 give a correlation of  $-.78$  between percentage germination and average days required for germination between phenotypes, and within phenotypes it is  $-.50$ . The regression of days on percentage for the former was  $-.085$  and  $-.046$  for the latter. The data on the analysis of covariance are shown in table 5. It is of some interest that the relationship is more pronounced between

the phenotypes than within them. The phenotype apparently has a significant influence on the relationship compared to other factors influencing it. This is thought to indicate a definite genetic relationship, otherwise the regression should be as great within phenotypes as it is between them.

TABLE 5

*Analysis of covariance for averages of means of percentage germination and the average means of the number of days required for germination of lines from backcross plants.*

| SOURCE OF VARIATION | DEGREES OF FREEDOM | PERCENTAGES    |          | DAYS           |          | COVARIANCE      |                         |                                   |
|---------------------|--------------------|----------------|----------|----------------|----------|-----------------|-------------------------|-----------------------------------|
|                     |                    | SUM OF SQUARES | MEAN SQ. | SUM OF SQUARES | MEAN SQ. | SUM OF PRODUCTS | CORRELATION COEFFICIENT | REGRESSION OF DAYS ON PERCENTAGES |
| TOTAL               | 92                 | 16797.0        | 182.58   | 168.0          | 1.83     | -1068.0         |                         |                                   |
| Between phenotypes  | 13                 | 7713.0         | 593.08   | 91.0           | 7.00     | - 653.0         | -0.779                  | -0.085                            |
| Within Phenotypes   | 79                 | 9084.0         | 144.99   | 77.0           | 0.97     | - 415.0         | -0.496                  | -0.046                            |

The effect of the *Ss* region on germination is thought to be related to the deficient number of recessives that occurred in the original backcross population. It will be recalled that in tables 1 and 2, the dominant group was approximately twice the size of the recessive group. Because the  $F_1$  plant was used as the pistillate parent in making the backcross, the possibility that differential pollen tube growth caused the disproportion in numbers is eliminated. Furthermore, LINDSTROM'S (1932) data shows approximately equal reduction in numbers of *s:S* whether the  $F_1$  was used as male or female for a similar backcross.

The following figures were extracted from table 2 and show quite clearly the deficiency in numbers mentioned above.

413 *D*: 257 *d* ratio of 1.6:1

409 *P*: 261 *p* ratio of 1.6:1

422 *O*: 248 *o* ratio of 1.7:1

447 *S*: 223 *s* ratio of 2.0:1

The excess of the *D*, *P* and *O* dominants is probably due to linkage with *S*. When the sizes of certain crossover phenotypic populations are considered, it is apparent that *s* consistently reduced the numbers and in various combinations of the other genes. An exceptional case is that the *D p o s* group contains 12 individuals and *d P O S* nine, but in all other pairings, the *S* group is in excess of the comparable *s* population. The *D P O s* group should equal *d p o S* but the numbers are 62:86. Likewise, *d p O S* contained 30 plants as against 17 for *D P o s*. (In both of these comparisons, there is an excess of recessives other than *s*.) It seems then, that the *s* region was acting as a partial lethal. The definite relation of



such an effect to time and percentage of germination is not known, but it is quite obvious that the lethal effect may not be more than a retarding or failure in germination of the seed without affecting the gametic ratio of  $S:s$ .

An earlier strain of  $d p o s$  was developed by selecting from the recessive backcross group and self pollinating the selections and again selecting for earliness. The selection was then crossed with the original  $F_1$ . After three repetitions of this program, the selected strain was compared with the  $d p o s$  parent for earliness, and was found to be several days earlier than the parent strain. Means obtained from the plants grown in adjacent rows were  $25.1 \pm 2.0$  for the parent strain and  $13.0 \pm 2.1$  for the selected strain. Inasmuch as the selected strain is significantly earlier, a possible reason would be the crossing over of earliness genes (independent of  $D P O S$ ) in the first chromosome. As an alternative explanation, it may be suggested that the selection program isolated genes for earliness in chromosomes other than the first. However, the former suggestion appears more probable, due to the indications in table 2 that earliness genes outside the first chromosome were not involved in the cross. It will be recalled that the uniformity of the  $D P O S$  and  $d p o s$  groups in the backcross was equal to that of the parents and no individuals in the  $D P O S$  phenotype were later than the early parent and none in the  $d p o s$  group were earlier than the late parent. The evidence therefore, is fairly satisfactory that there are genes for earliness in the chromosome that cross over independently of the four marker genes.

#### THE EFFECT OF POLYPLOIDY ON EARLINESS

The data on this are not extensive but may be of some significance. By the decapitation method, a shoot that had all the external characteristics of a tetraploid was produced on an  $F_1$  plant. Seeds were obtained from this branch as well as from a normal branch of the same plant. When the plants resulting from these two lots of seed were compared for earliness, the two means were almost identical. The plants were distributed over the ripening period as shown in table 6.

TABLE 6  
*Comparison of ripening dates for 4n and 2n  $F_2$  populations.*

|            | RIPENING DATES |   |    |    |    |    |    |    |    |    |    |    | TOTAL | MEAN           |
|------------|----------------|---|----|----|----|----|----|----|----|----|----|----|-------|----------------|
|            | 0              | 4 | 8  | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 |       |                |
| Diploid    |                | 3 | 2  | 6  | 1  | 1  | 1  |    |    |    |    |    | 14    | $7.4 \pm 3.67$ |
| Tetraploid | 1              | 9 | 11 | 14 | 2  | 4  | 3  | 5  | 1  | 1  | 0  | 1  | 52    | $7.8 \pm 2.38$ |

Although the above comparison is somewhat inadequate, there are indications that earliness of the plants is not increased by increasing the number of chromosomes carrying genes for earliness. The effect of the genes that delay fruiting may be increased since the three latest plants in table 6 were recessive and were later than any of the diploid plants. The effect of polyploidy is being studied further.

## SUMMARY

1. Chromosome 1 was found to have an effect of approximately 19 days on time of fruiting.
2. Differences in time of fruiting associated with the *Dd* region of the chromosome were about eight days. With the *Pp* region, the difference in time was 5 days. For the *Oo* region there was no definite association. The *Ss* region gave differences varying from 4 to 8 days. The nature of the interaction exhibited was such that genes for earliness tended to reduce the effect of other earliness genes. Definite indications of such an interaction was shown by the *Ss* region.
3. The effect of different parts of the chromosome on earliness was not related to crossover lengths of the regions.
4. The different regions were noted to have an effect very early in the life cycle since the time required for germination and the percentage of germination were affected. The *Ss* region was distinctly effective in this respect and this associated with the deficient number of recessives that were obtained in the backcross population.
5. By repeated backcrossing and selecting for early *dd pp oo ss*, a strain was developed that is significantly earlier than the *d p o s* parent. This suggests that genes other than *d p o s* in the first chromosome affect rate of development.
6. A tetraploid  $F_3$  population appeared to be somewhat more variable for earliness than a comparable diploid population, but increasing the number of chromosomes containing earliness genes did not produce earlier plants.

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# INHERITANCE OF CERTAIN "BLUE-BLACK" PATTERNS AND "BLEACHED" COLORATIONS IN THE DOMESTIC PIGEON<sup>1</sup>

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Received August 17, 1937

## INTRODUCTION

TWO objectives are concerned in the following study: to clear up as far as possible the questions concerning the inheritance of certain "blue-black" patterns previously discussed by JONES (1922) and by BOL (1926), and to analyze several previously unrecognized color types.

The coloration of the ordinarily accepted wild type of *Columbia livia* is here used as a standard of reference. The plumage is basically a light bluish gray; the remiges become black distally, and the ends of the rectrices also show black distally but in the form of a broad band with a faint bluish terminal edge. The tertiary wing feathers exhibit a black area in the vanes, as do the secondary coverts and often the median coverts; these rows of black areas form two black bars across the closed wing. The neck and crop feathers are dark, with much iridescence. The rump and underwing feathers are albescent or whitish terminally; the outer vanes of the outer rectrices are also albescent. The iris of the eye is red at the margin and more yellow centrally, giving an orange appearance. The beak and claws are very dark brown or black.

A list of factors incidentally involved in the present study and descriptions in terms of deviations from wild type follow. Wild type factors are symbolized "+."

*d*—"dilution"; sex-linked. Nestling down short, fine; beak, claws, and skin pale; retinal pigmentation less intense; plumage tawny with dun pattern. The symbol *i* has also been used for this factor.

CHRISTIE and WRIEDT (1923).

*B*<sup>4</sup>—"ash"; sex-linked. Plumage ashy gray with reddish brown neck, crop, and wing pattern. Also has been termed "dominant red" and "A-factor." HAWKINS (1931).

*b*—"chocolate"; sex-linked, allele of *B*<sup>4</sup>. Beak, claws, and skin pale; iris less yellow; plumage tawny with brown pattern. STEELE (1931) and HAWKINS (1931).

*e*—"recessive red"; beak, claws, and skin pale; plumage more or less uniformly chestnut red, least effect on albescent areas and in proximal part of tail. STEELE (1931).

<sup>1</sup> Paper from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 220. Published with the approval of the Director of the Station.

S—"spreading"; plumage dull black, usually with wing bars still faintly visible. JONES (1922).

G—"grizzle"; finely stippled decolorization (whitening) in plumage, least in distal parts of remiges and rectrices. BOL (1926).

Studies of feather development in general show that the source of pigment is mainly large chromatophore cells, whose branching pseudopodia deposit pigment in the cornifying structures. These chromatophores appear to arise in the growth ring at the root of the feather germ. Microscopic examination of the pigment left in the mature feather of the wild *C. livia* has shown that only one kind of pigment, black, is present. The

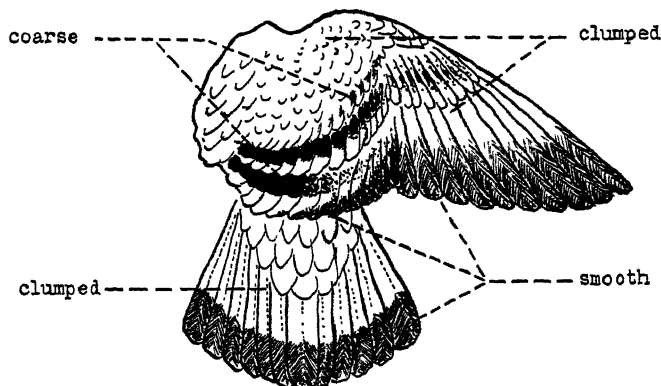


FIGURE 1.—Diagram of wild type color pattern, showing the positions of the clumped and spread phases of the pigment.

appearance of "blue" portions of the plumage under the microscope resembles a half-tone print, each barbule showing striations or clumping of black granules. The black portions of the plumage reveal only traces of the clumping or striation, if any, the clumps being so wide as to coalesce. These two phases of the blackish pigment have been called "clumping" and "spreading" respectively (LLOYD-JONES 1915).

Two slightly different sorts of "spreading" should be recognized. One of them involves the distal portions of the remiges and the tail band, while the other affects the wing bars (fig. 1.) Examination of these areas of a wild type specimen reveals in the first case a smooth transition from black to blue, while in the second case there are usually slight irregularities and "freckling" in the transition. For convenience the two types of spreading will be referred to as the "smooth" and the "coarse" types, respectively. There is apparently no microscopic difference, but as will be seen further on, the two react differently in many color combinations. The effect of S seems to be chiefly to cause deposition of pigment of the "smooth" spread type throughout the plumage.

The tendency for spreading of pigment to occur in specific areas of the plumage and individual feathers results in patterns. A wide variety of such patterns is found in the Columbidae, and has furnished ground for voluminous speculations on evolutionary relationships (WHITMAN 1919). Within the limits of domestic breeds also a remarkable range of pattern may be observed.

#### "BLUE-BLACK" PATTERNS

Fanciers and most students of pigeon inheritance have adopted a simple system of "blue-black" pattern nomenclature based on the modal types, namely barless, bar (wild type), and checker. Appropriate adjectives designate deviation from these modal conditions. The checker pattern has been subdivided according to the amount of spreading into several sub-types; extremely dark checker has been termed the "T" type. Another condition which JONES (1922) named "sooty" has often been lumped with checker. Specimens showing the various patterns are well illustrated in the works of WHITMAN, JONES, and BOL.

The patterns differ from each other only in the amount and position of coarse spreading. The barless pattern lacks it almost entirely; the other patterns show in serial array increasing invasion in the wings, the crop, and finally nearly all the body feathers anterior to the tail.

JONES considered the patterns "barless," "bar" (wild type), "sooty," "checker," and "T" as independently inherited but forming an epistatic series. BOL considered them to be alleles. Neither gave due treatment of intermediate conditions, and the evidence for their respective hypotheses is insufficient.

From the above studies we can accept two conclusions: that despite a perfectly graduated series of patterns, sharp segregations occur frequently; and that in general dominance is parallel to the amount of "spreading" involved in the pattern.

A restudy of the problem has been made. It seemed obvious at the start that the "sooty" type is not homologous with the other patterns, since "spreading" occurs along the shaft and end of affected feathers. In other patterns spreading encroaches from the side toward the shaft. Since no satisfactory source of sooty was available at the time, this type has not been studied further.

Breeding data obtained in the study of the patterns are summarized in table 1. Barred birds, of several color classes, and chiefly of Homing pigeon ancestry, have been used as the wild type pattern. In all cases they have been typical, but not entirely alike. A family of barless Strassers and a checker Strasser cock whose dam was barless constituted the source of the barless character.

Two matings of barless male by bar female gave 16 offspring, all barred. Some tendency to narrowness and shortness of the bars was observable. Three matings of the  $F_1$  produced 47  $F_2$ ; of these only seven were barless. In the others the character of the barring was decidedly variable, the bars being mere rudiments in two cases. That a single factor differentiates bar-

TABLE I

*Results of matings involving T-pattern, checker, bar, and barless.*

| MATING  | PHENOTYPE OF OFFSPRING |     |     |    |
|---|------------------------|-----|-----|----|
|   | $C^T$                  | C   | +   | c  |
| Barless $\times$ ++ (bar)   |                        |     | 16  |    |
| $F_1 \times F_1$  |                        |     | 40  | 7  |
| T pattern (origin 2380F) $\times$ ++                                      | 33                     |     | 31  |    |
| T-pattern (origin 2707.1) $\times$ ++                                     | 4                      |     | 6   |    |
| Medium checker (origin 7-29-1) $\times$ ++                                |                        | 117 | 131 |    |
| Checker, heavy grade (origin 2267N) $\times$ ++                           |                        | 25  | 19  |    |
| Checker, light grade (origin 2835.1) $\times$ ++                          |                        | 7   | 5   |    |
| T-pattern (origin 2380F) $\times$ barless                                 | 3                      |     | 6   |    |
| $F_1$ T-pattern $\times$ barless  | 18                     |     |     | 17 |
| T-pattern $\times$ checker (medium, heterozygous for bar)                 | 8                      |     |     |    |
| $F_1$ (T-pattern) $\times$ ++   | 27<br>8                | 25  | 10  |    |
|   | 35                     | 35  |     |    |
| Checker (origin 8-33-14) $\times$ barless                                 |                        | 6   |     | 4  |
| Checker (origin 8-33-14) $\times$ ++                                      |                        | 3   | 6   |    |
| $F_1$ bar $\times F_1$ bar  |                        |     | 9   | 1  |
| T-pattern (origin 2380F) $\times$ checker (medium, heterozygous for bar)  | 23                     | 10  | 6   |    |
| T-pattern (origin 2707.1) $\times$ checker (medium, heterozygous for bar) | 3                      | 1   | 1   |    |
| $F_1$ T-pattern $\times$ ++   | 24                     |     | 26  |    |
| Medium checker $\times$ ++  |                        | 32  |     |    |
| $F_1 \times ++$   |                        | 19  | 19  |    |

less from barred is not excluded by these facts, but the presence of modifying factors must be considered.

The backcross method is more suitable for analytical purposes and has been used in most of the remaining work. Two birds of unrelated stock form the sources of all T-pattern birds used: one, 2707.1, was heterozygous for bar; mated with bar he gave ten squabs, four T-pattern and six bar. The other, 2251F, was not heterozygous for bar, since in a mating (2380) with a bar female none of the eight offspring were barred. A daughter, 2380F, was mated with a bar male. Of sixteen offspring nine were T and seven bar. Some of the former were crossed with bar, and the process

repeated for two more generations. Of a total of 64 offspring so produced, 33 were T-pattern or very heavy checker, and 31 bar. No definite distinction between T-pattern and very heavy checker seemed feasible, and since on a two-factor basis a ratio of 48:16 would be expected, it seems clear that T-pattern and very heavy checker are, in this case at least, slightly different expressions of one dominant factor.

In a similar study three checkered birds were sources of separate families. All three birds were heterozygous for bar. One was a heavy grade male, 2267N; another was a medium heavy grade female, 7-29-1; the last was a very light grade male, 2835.1. In each family approximate equality of checker and bar descendants was obtained. The factor for checker in each case then behaved as a simple dominant. But instead of a regression to a common modal condition for all families, it was found that the condition in each family was similar to that of its source bird. The family derived from 7-29-1 was the largest and most varied; the modal condition here was medium grade, but some individuals were fairly light, and a few even heavier than the source bird.

The conclusion is that a different chief factor or allele for checker was introduced by each source bird, but that modifying factors are capable of shifting the grade of the checker to some extent, as in the case of the T-pattern. At the extremes of this variation the phenotype of one factor may overlap that of the next higher or lower grade factor and the highest may even be confused with T-pattern. It is advisable then to use breeding tests to classify intermediate individuals of unknown origin.

To determine whether the more distinct types (barless, medium checker, and T-pattern) might be allelic, tests were made as follows. A female, 2670J, T-pattern (heterozygous for bar) and descendant of 2380F, was crossed with a barless male Strasser. Nine offspring resulted—three very heavy check and six barred. The former were back-crossed to barless Strasser, producing 18 very heavy check and 17 barless. Barless and the T factor from 2380F are therefore alleles. The male 2251F (homozygous T) was crossed with a medium grade checker heterozygous for bar. Seven offspring (all T-type) were mated to bar. Five produced 52 offspring, of which 25 were medium grade checker and 27 T-pattern or very heavy checker. The other two produced a total of eighteen young, of which ten were barred and eight T-pattern or very heavy check. Therefore the T factors from 2251F are allelic with the medium-grade checker factor.

Only one mating was made to test the allelism of checker with barless. The checkered Strasser 8-33-14, whose dam was barless, was mated with barless. Of the ten offspring, six were checker and four barless.

To sum up: T-type, heterozygous, by other types gave 143 T:139 others. Checker, heterozygous, by bar or barless gave 178 C:187 others.

Bar, heterozygous for barless, inbred, gave 49 bar:8 barless (expectation 43:14).

In all tests allelism of these three patterns is found. There is little reason to suspect a different mode of inheritance in the other cases although they have not been tested.

The above findings explain the results of a number of other available matings. Two matings of T-type, heterozygous for bar, by medium grade checker, also heterozygous for bar, gave 26 T:11 checker:7 bar (expectation 22:11:11). Two T-type offspring crossed to bar, gave a total of 50:

TABLE 2A  
*Results of matings involving opal.*

| MATING  | PHENOTYPE OF OFFSPRING       |                        |     |      |
|---|------------------------------|------------------------|-----|------|
|   | INFERTILE,<br>DEAD<br>EMBRYO | DEAD<br>YOUNG,<br>ETC. | +   | OPAL |
| Opal×opal                                       | 136                          | 87                     |     | 261  |
| Opal male×++female                              | 11                           | 16                     | 113 |      |
| ++male×opal female                              | 25                           | 19                     | 20  |      |
| Opal male×F <sub>1</sub> female (or equivalent) | 63                           | 100                    | 300 | 258  |
| F <sub>1</sub> male (or equivalent)×opal female | 115                          | 57                     | 133 | 134  |
| F <sub>1</sub> ×F <sub>1</sub> (or equivalent)  | 8                            | 10                     | 49  | 21   |
| Totals:   |                              |                        |     |      |
| in outcross                                     |                              |                        | 133 |      |
| in back-cross                                   |                              |                        | 433 | 392  |

24 T and 26 bar. Male 8-33-14, checker heterozygous for barless, when mated with a bar gave nine young—three checker and six bar. The latter barred birds carry the barless factor. Two of them were inbred, and did produce a barless squab, but as in the previous case of barred carrying barless, the ratio is poor (9 barred:1 barless), and variability of the barring was marked. Homozygous checker 8-33-6 mated with bar produced 32 offspring, all checker. No marked difference between the homozygote and most of his heterozygous offspring has been found. These in turn mated with bar gave a total of 19 checker and 19 bar.

The symbolization of the factors in this allelic series as suggested by JONES and by BOL seems at present undesirable. DE HAAN's (1933) method will be followed in general. Barless will be *c*, checker *C*, and T-pattern *C<sup>T</sup>*. The dominance relations in the series may be represented as follows: *C<sup>T</sup>* > *C* > + > *c*.

#### "BLEACHED" COLORS

The "bleached" appearance of the "ash" (*B<sup>A</sup>*) plumage is imitated to some extent by four new factors. These have been given the names "opal,"



"dominant opal," "faded," and "milky." Experience and the use of suitable breeding methods are necessary in differentiating these types from each other as well as from  $B^4$ .

### *Opal*

"Opal" seems widely distributed in Homing pigeons, since it has been obtained or observed by the writer in many separate flocks. Probably WHITMAN (1919) was dealing with it in his chapter on "Color and Weakness in Homers" (vol. II, chap. IX). Figures representing the condition are given in his vol. I, plate V, A and B. WHITMAN considered the cause of any such color to be low vigor in one or both parents.

In appearance most opal birds are faded black to grayish ashy in the regions of "smooth" spreading; the remiges generally show less "bleaching" than the tail band. The "coarse" spread areas generally exhibit a distal edge which is blackish, while proximally a narrow ashy line and then reddening are found. Clumped areas of the wing plumage are practically normal (blue) except in the more extreme cases; in these the clumped areas are bleached out also, resembling the condition found in  $B^4$ . The blue of the rectrices and tail coverts, however, becomes bleached to some extent in all cases.

Breeding data involving opal are given in table 2a. Since in outcrosses no opals were produced the character is recessive; in  $F_2$  it occurred in a frequency of 30 percent, with standard deviation of 5.47 percent. In the backcross the opals are slightly in the minority but chance can easily account for the discrepancy. And finally, opals breed true. The inheritance is therefore definitely monofactorial; the symbol  $o$  will be used.

Variability in the degree of "bleaching" in opal birds is most striking. Almost every case shows slight banding of the feathers, representing variation in effect at different times while the pigment is being deposited. Definite "banding" is frequent, and easily induced by starving. The starvation period is marked by a very light bar across the feathers. RIDDLE (1908, p. 357) states that "pale-colored" pigeons are most suitable for studies of "fundamental bars." Opal birds were observed by the writer in RIDDLE's "family 133." At the time, RIDDLE commented that such a color type was referred to by the term "pale-colored."

Opals sometimes also exhibit marked change of color at different molts. Since such variation seemed due to physiological states, a test was made to see whether thyroid feeding would have similar effect. A male with little "bleaching" was chosen, 2652F. Secondary covert feathers were removed from both wings. Five days later, just before the new quills came through the skin, feeding of capsules of thyroid powder (Armour's, 0.2 percent iodine) was started. Two doses a day of .15 gram each were

given over a period of five days. By this time the feathers were well out of the skin and pigmentation nearly complete. These feathers were much more bleached than the previous set; the new ones were removed two weeks after cessation of thyroid feeding. The next regeneration (no thyroid feeding) grew in dark again.

In another case the same total amount of thyroid (1.5 grams) was given to a dark opal male, 2730D, in one dose. Apparently its effect wore off before pigmentation of new feathers began, since they were dark.

If the first case above is to be considered significant, it may be surmised that the activity of the birds' own thyroid is correlated with the variability

TABLE 2B

*Reproductive behavior of females in opal matings. Standard deviations given.*

| TYPE OF FEMALE | TOTAL EGGS | INFERTILE?,<br>DEAD<br>EMBRYO | BROKEN EGGS,<br>DEAD<br>YOUNG | SURVIVORS   |
|----------------|------------|-------------------------------|-------------------------------|-------------|
|                |            | %                             | %                             | %           |
| Normal         | 964        | 8.6 ± .9                      | 13.0 ± 1.08                   | 78.4 ± 1.32 |
| Opal           | 956        | 22.1 ± 1.11                   | 15.3 ± 1.16                   | 62.6 ± 1.57 |
| Extreme opal   | 122        | 53.3 ± 4.5                    | 13.1 ± 3.0                    | 33.6 ± 4.3  |

of opal. Just how much of the variation can be accounted for on this basis is questionable since the "extreme" opals (closely resembling  $B^4$ ) do *not* show any fluctuation at different molts or in the individual feathers. The possibility that the extreme condition is due to another opal allele has no genetic support at present.

Opal females have been decidedly poor breeders, as is shown in table 2b. The chief point of trouble seems to be the egg; no matter what kind of male is involved, apparent infertility and embryonic death is considerably greater than in eggs from females of other colors. It seems unlikely therefore that the genotype of the embryo is responsible. Further evidence in this direction is afforded by the fact that once hatched, opals and other squabs seem equally hardy, and that the segregation ratios are approximately correct for a simple recessive.

Opal has been found closely linked with the locus of the pattern series treated earlier in the paper; the details of this study are to be published separately.

#### *Dominant opal*

A coloration known to fanciers as "white bar" or "white checker" (according to the pattern) in the Strasser breed greatly resembles the ordinary opal, though less reddish appears in the wing pattern: also, the bleaching is often more obvious in the primaries than in the case of recessive opal.

Illustrations of Strassers of this color may be seen in WITTIG part VI, pp. 33-37.

One male Strasser of this type, 8-33-14, has been the source of the type in this study. The breeding data are summarized in table 3. No inbreeding was practiced, and therefore no homozygotes produced. About half the offspring of affected parents were of the type; a female (2694D) produced daughters of the color. The trait is then dominant and autosomal, and will be termed "dominant opal," with the symbol *Od*.

This same female, 2694D, being the daughter of a recessive opal dam, is heterozygous for *o*. Backcrossed to an opal male, she gave 22 offspring; among them was one wild type, and six were apparently the combination of both opal types. Dominant opal is therefore not an allele of recessive opal. The expression is not nearly so variable as in the case of recessive opal. Occasionally a few small reversionary (blue or black) flecks have been observed in the plumage.

#### *Faded*

A third "bleached" coloration type was kindly given the writer by Dr. H. W. FELDMAN from the pigeon colony at the University of Michigan. The ancestry was Tippler  $\times$  Parlor Tumbler; probably the latter was the actual source of the trait, but the coloration has not been given a name by fanciers. The phenotype resembles a low-grade opal in many respects, but the clumped areas are more definitely bleached. The plumage of both sexes shows frequent reversionary (blue or black) flecks.

One male, 2707.1, was used. The procedure followed was the same as in the case of dominant opal, repeated outcrosses, and testing for allelism with recessive opal. Here again dominance was demonstrated. As yet no females have been bred, so that whether the trait is sex-linked or not is unknown. At any rate the factor is not an allele of recessive opal; it will be given the name "faded," and the symbol *Of*. A summary of the breeding data is given in table 3.

#### *Milky*

The last type of "bleached" coloration to be discussed here has been given the name "milky." The origin was a single male street pigeon, 8-33-15; no other sources of the trait are yet known. It has proved very uniform in phenotype, the "bleaching" always being quite pronounced, with little reddening in the "coarse" spread pattern.

The original male was crossed with a blue and with an opal, respectively; all progeny were blue. He was mated with a daughter, and produced eight young, only one of which was milky. Matings of the  $F_1$  however, gave a fair 3:1 segregation (table 3). No other information is available on this

trait, but it seems safe to assume that a simple recessive autosomal factor is responsible, not an allele of opal; the symbol *my* will be used.

### *Smoky*

"Smoky" plumage color is well known in several breeds of pigeons and occurs sporadically in many others. In this study the trait has been introduced by several Homing pigeons and by the Archangel breed. Smoky

TABLE 3  
*Results of matings involving dominant opal, faded, milky, and smoky.*

| MATING  | PHENOTYPE OF OFFSPRING |               |           |          |
|---|------------------------|---------------|-----------|----------|
|   | DOM. OPAL              |               | +         |          |
|   | 27                     |               | 30        |          |
| Dominant opal×++                              | <i>Od</i>              | ( <i>Od</i> ) | +         | <i>o</i> |
|   |                        | <i>o</i>      |           |          |
| Dom. opal×recessive opal                      | 3                      |               | 4         |          |
| F <sub>1</sub> dom. opal×rec. opal            | 6                      | 6             | 1         | 9        |
|   | Faded                  |               | +         |          |
|   | 16                     |               | 10        |          |
| Faded×++                                      | 2                      |               | 3         |          |
| Faded×recessive opal                          |                        |               |           |          |
|   | <i>Of</i>              | ( <i>Of</i> ) | +         | <i>o</i> |
|   |                        | <i>o</i>      |           |          |
| F <sub>1</sub> faded×recessive opal           | 5                      | 5             | 6         | 4        |
|   | +                      |               | <i>my</i> |          |
| Milky×++                                      | 9                      |               |           |          |
| Milky×recessive opal                          | 7                      |               |           |          |
| F <sub>1</sub> ×F <sub>1</sub>                | 23                     |               | 9         |          |
| F <sub>1</sub> ×milky                         | 7                      |               | 1         |          |
|   | +                      |               | <i>sy</i> |          |
| smoky×++                                      | 78                     |               |           |          |
| F <sub>1</sub> (or equivalent)×F <sub>1</sub> | 148                    |               | 46        |          |
| F <sub>1</sub> (or equivalent)×smoky          | 44                     |               | 45        |          |
| Smoky×smoky                                   |                        |               | 78        |          |

birds have light skin and base of beak, especially noticeable in squabs; the wing pattern is blurred through a darkening of the blue portion and less richness of the "coarse" spreading. The bluish tip edges of the rectrices are accentuated, and the albescence under the wings, on the rump, and in the outer rectrices is replaced by slaty blue.

This group of features was found to breed true in all cases. Other breeding data (table 3) show that a single factor is responsible for this syndrome. In the heterozygous condition the beak is slightly light, and there may be slight loss of albescence, or accentuated blue-tipping of the rec-

trices, but the trait may best be treated as a recessive. It is symbolized *sy*.

Modern Carriers, Barbs, and Magpies seem all to be smoky, according to show standards and numerous examples which the writer has observed. In addition, the ordinary varieties of Archangel seem always smoky.

STAPLES-BROWNE (1908) used the Barb in some of his crosses. Two "blue" segregates are pictured in his paper (plate V); the one on the left, except for beak color, which may have been darkened by sunlight, seems a typical smoky in the T-pattern.

#### COMBINATIONS

Despite the fact that all the eight factors described have to do with only one general process, namely pigmentation, most of the combinations which have been obtained have not usually been difficult to detect phenotypically. Generally the phenotype may be predicted from knowledge of the effects of the single factors.

In combinations of *C* or its alleles with *S* and with *e*, the latter are epistatic.

Combinations of recessive opal with *B<sup>A</sup>*, *b*, *d*, and *e* have generally been difficult to classify as opal without breeding tests, but many individuals show unmistakable bleaching.

The combination of recessive with dominant opal resembles the dominant opal mainly, but also possesses certain features of the recessive opal which are recognizable with experience.

In the combination of recessive opal with faded, a more or less additive effect is seen; the general appearance is recessive opal with marked bleaching of the clumped areas as well.

Smoky in combination with *S* gives a rich, even black; the base of the beak is light. It is very easy to confuse the homozygous and heterozygous conditions, and it is safest to classify such specimens by breeding test.

Smoky is even more difficult to recognize in combination with *e*; the lack of typical albescence is characteristic, especially in the rectrices, but the phenotype is mainly that of *e*.

#### SUMMARY<sup>2</sup>

A series of at least three alleles has been shown to be concerned in the inheritance of the patterns involving the extent of one phase of pigmentation. These are *c* (barless), *C*, (checker, medium grade), and *C<sup>T</sup>* (T-pattern and very heavy checker). The variability in expression of *C* and *C<sup>T</sup>* seems to be due to modifying factors. Other alleles are indicated but not proved to exist; variability hinders their recognition.

<sup>2</sup> Detailed data have been filed in the editorial office of GENETICS and may be consulted by anyone interested; they are also to be found in the writer's thesis (University of Wisconsin, 1937).

Four factors responsible for "bleached" phenotypes somewhat similar to that produced by the sex-linked  $B^A$  factor have been identified. Opal and milky are recessives, while Dominant opal and Faded are dominants. The first three are autosomal; Faded has not been tested for sex-linkage. Opal is not an allele of the other three; it is closely linked with  $C$  and its alleles. Furthermore, poor hatchability has been observed with eggs produced by females of the opal type. Opal is the most variable of the four factors studied; a large share of the variability seems the result of metabolic variability during feather growth.

The color modification smoky has been found to be a simple autosomal recessive; it is apparently a characteristic color feature of several breeds.

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# A SEX DIFFERENCE IN LINKAGE INTENSITY OF THREE AUTOSOMAL FACTORS IN THE DOMESTIC PIGEON<sup>1</sup>

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Received August 17, 1937

**D**URING a study of color inheritance in the domestic pigeon three autosomal factors have been found to be linked. In a preliminary report (HOLLANDER 1936) certain statements were made which must be modified in the light of additional data.

Previous authors have failed to demonstrate any autosomal linkages in the pigeon. CHRISTIE and WRIEDT (1923) attempted to show a linkage between white-tail and saddle or shield pattern, but the characters were not proved simple and the numbers were too small for significance. Crossing-over between sex-linked factors has been reported by COLE and KELLEY (1919), CHRISTIE and WRIEDT (1925), STEELE (1931), and HAWKINS (1931).

JONES (1922) gave results of ten backcross matings involving the segregation of black (*S*) and checker (*C*); the characters are both dominant to the wild type or "blue bar." From the ten matings 73 offspring were obtained; of them, 41 lacked *S*. Since the *S* factor is epistatic to *C*, only these 41 can be utilized in estimating a possible linkage. A review of the ancestry of the heterozygotes used in the matings has shown that all are in the coupling phase. There appears to be a suggestion of linkage: female heterozygotes produced six checker and 13 bar progeny, and males nine checker and 13 bar.

The same factors have been used in the present study. In addition, the T-pattern, a dominant allele of checker (*C'*) and a third factor, "opal" color (*o*) have been included (HOLLANDER 1938).

A few other color factors have occasionally come into the tests. These are *B*<sup>4</sup> (ash, sex-linked); *b* (chocolate, a recessive allele of *B*<sup>4</sup>); *d* (dilution, sex-linked); *e* (recessive red); *G* (grizzle); *Od* (dominant opal, not an allele of *o*); *Of* (faded); and *sy* (smoky). The combinations of the color factors which were encountered were seldom confusing. However, epistasis of ash, dilution, and recessive red over opal, and of recessive red over *S* and *C*, necessitated leaving a few birds unclassified, except when further breeding tests have revealed their constitution (HOLLANDER 1938). The results may be summarized as follows:

<sup>1</sup> Paper from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 221. Published with the approval of the Director of the Station.

| REGION     | MALE HETEROZYGOTES |             |                  | FEMALE HETEROZYGOTES |             |                  |
|------------|--------------------|-------------|------------------|----------------------|-------------|------------------|
|            | NON-CROSS-OVERS    | CROSS-OVERS | %<br>CROSS-OVERS | NON-CROSS-OVERS      | CROSS-OVERS | %<br>CROSS-OVERS |
| $S-C(C^T)$ | 28                 | 23          | $45.1 \pm 7.0$   | 83                   | 13          | $13.5 \pm 3.5$   |
| $S-o$      | 19                 | 23          | $54.8 \pm 7.5$   | 102                  | 19          | $15.7 \pm 3.3$   |
| $C(C^T)-o$ | 99                 | 7           | $6.6 \pm 2.4$    | 251                  | 6           | $2.3 \pm .9$     |

Male heterozygotes have given definite evidence for linkage only in the case of  $C$  with  $o$ , while females show definite linkage in each test. The figures are so significant that we are driven to conclude that a sex difference in crossing over exists.

Three-point tests have also been made. Analysis into the component two-point data is summarized as follows:

| REGION     | MALE HETEROZYGOTES |             |                  | FEMALE HETEROZYGOTES |             |                  |
|------------|--------------------|-------------|------------------|----------------------|-------------|------------------|
|            | NON-CROSS-OVERS    | CROSS-OVERS | %<br>CROSS-OVERS | NON-CROSS-OVERS      | CROSS-OVERS | %<br>CROSS-OVERS |
| $S-C(C^T)$ | 29                 | 21          | $42.0 \pm 7.0$   | 57                   | 12          | $17.4 \pm 4.6$   |
| $S-o$      | 49                 | 38          | $43.7 \pm 5.3$   | 100                  | 25          | $20.0 \pm 3.6$   |
| $C(C^T)-o$ | 43                 | 4           | $8.5 \pm 4.1$    | 67                   | 2           | $2.9 \pm 2.0$    |

These values are very similar to those from the two-point tests. When all the data are combined, values are obtained as follows:

|            |     |    |                |     |    |                |
|------------|-----|----|----------------|-----|----|----------------|
| $S-C(C^T)$ | 57  | 44 | $43.6 \pm 4.9$ | 140 | 25 | $15.2 \pm 2.8$ |
| $S-o$      | 68  | 61 | $47.3 \pm 4.4$ | 202 | 44 | $17.9 \pm 2.4$ |
| $C(C^T)-o$ | 142 | 11 | $7.2 \pm 2.1$  | 318 | 8  | $2.5 \pm .9$   |

The factorial order indicated by the above values is  $S, C, o$ , although the difference between  $S-o$  and  $S-C$  is so small that it is not in itself statistically significant. However, there is another basis for assuming the same order. In the three-point tests one crossover class was contributed to only by male heterozygotes; this probable double crossover class also indicates the factorial order  $S, C, o$ .

In figure 1 chromosome maps based on the order assumed and utilizing the total crossover frequencies for  $S-C$  and  $C-o$ , separately for each sex of heterozygote, are shown. It is quite likely that the actual map distances shown are too short in the region  $S-C$ , because of double crossing over,



but the positions of the factors can only be marked approximately at best.<sup>2</sup>

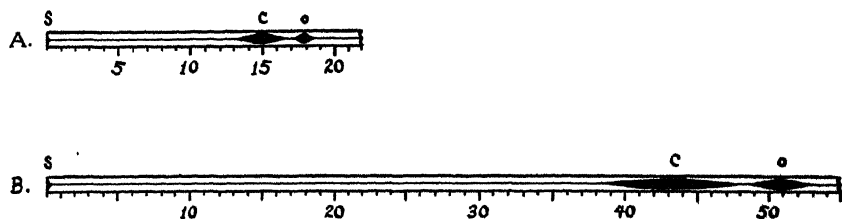


FIGURE 1. Sketches of the probable order and map distances, A. from female heterozygotes, and B. from males.

#### DISCUSSION

The high rate of embryonic death in many of the matings of male heterozygotes deserves comment. The high crossover percentages in males cannot be explained by differential mortality since opal females, also involved in such matings, have been found to give eggs of poor hatchability much more frequently than other females, no matter what type of male is used (HOLLANDER 1938).

From the linkage data it is clear that in the case of this autosome, female heterozygotes show between one-fourth and one-third as much crossing over as males. Whether this is true for the other autosomes is as yet unknown; in other organisms which have been investigated, the chromosomes are fairly consistent with each other in this respect.

The pigeon might be expected to resemble the fowl in its sex difference of linkage intensity, but such is not the case according to data at present available. The male fowl has slightly less crossing over than the female (WARREN and HUTT 1936). *Drosophila* ordinarily shows no crossing over in the male, while the silkworm moth is reported to have none in the female (STURTEVANT 1915). Mice and rats show somewhat less crossing over in males than in females (GATES 1931). The pigeon then seems to stand alone among animals whose linkage phenomena have been studied in its marked reduction of crossing over in the female.

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<sup>2</sup> Tables of detailed data have been deposited in the editorial office of *GENETICS* and may be consulted by anyone interested.

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# INVERSIONS IN THE CHROMOSOMES OF *DROSOPHILA PSEUDOOBSCURA*\*

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Received August 23, 1937

## INTRODUCTION

THE linear arrangement of genes within chromosomes is constant from generation to generation in each line of descent. The degree of this stability is comparable to that of the gene structure; genes change by mutation, chromosomes change by the occurrence of chromosomal aberrations. Strains and races of the same species, as well as distinct species, may differ in gene arrangement. One of us (STURTEVANT 1917, 1931) has shown that strains of *Drosophila melanogaster* coming from the same or from distinct geographical localities may differ in having blocks of genes rotated by  $180^\circ$  (inversion). The method of detection of inversions used in these early studies was based chiefly on observations on the strength of linkage in hybrids between different strains, but the laboriousness of this method limits its applicability. The discovery of the salivary gland chromosome method has rendered the task much easier. TAN (1935) in *Drosophila pseudoobscura*, and DUBININ, SOKOLOV and TINIAKOV (1936) in *melanogaster* have observed numerous inversions in hybrids between strains and races. DOBZHANSKY and TAN (1936) and DOBZHANSKY and BAUER (unpublished) have found extensive differences between the gene arrangements in two pairs of species of *Drosophila*, namely *pseudoobscura* and *miranda*, *athabasca* and *azteca*.

In the present article we shall report the results of comparisons of the gene arrangement in the chromosomes of strains of *D. pseudoobscura* coming from different geographical regions. This species has five pairs of chromosomes; one of them, the third, is especially variable in the gene arrangement. Other chromosomes are relatively more constant, yet some variation has been observed in every chromosome, except the small fifth. Some of the gene arrangements are encountered in populations inhabiting a major part of the species area, while others are more restricted in their distribution. In many localities the population is mixed with respect to the chromosome structure, and inversion heterozygotes are very common in nature. Moreover, as pointed out in our preliminary communication (STURTEVANT and DOBZHANSKY 1936a), a comparison of the different gene arrangements in the same chromosome may, in certain cases, throw light on the historical relationships of these structures, and consequently on the history of the species as a whole.

\* The cost of Plate 1 is paid for by the Galton and Meudel Fund.

## MATERIAL AND METHODS

In the salivary gland cells of many Diptera, including *Drosophila*, the homologous chromosomes undergo a very intimate pairing. It is now clear that this pairing is due to a mutual attraction between the homologous loci in the chromosomes rather than between chromosomes as wholes. Two chromosomes containing the same genes arranged in identical linear orders fuse to form a double strand in which homologous loci lie on exactly the same levels. Pairing of chromosome sections having dissimilar disc patterns (and consequently dissimilar genes) is never observed, at least in our species. If the gene arrangements in two chromosomes are not identical, the homologous discs still tend to become associated, forcing the chromosomes to form at times very complex pairing configurations. For example, if two homologous chromosomes have a section inverted, a loop-like configuration appears in the salivary gland cells (fig. 1).

This chromosome pairing furnishes an easy and accurate method for comparison of the gene arrangements in different strains of the same species, or in different species if these can be crossed. A strain is selected the gene arrangement in which is arbitrarily chosen as a standard. Strains to be tested are crossed to the standard one, and the chromosomes are examined in the salivary glands of the larvae of the first generation hybrids. If the strains crossed are identical with respect to gene arrangement, all the chromosomes in the hybrids are represented by paired strands radiating from the chromocenter. If the gene arrangements are different in any respect, some of the chromosomes in the hybrids show abnormal pairing configurations, from the appearance of which the precise nature of the difference can be deduced.

The procedure just outlined has been followed in the present investigation. A strain of race A of *D. pseudoobscura* carrying the third-chromosome recessives orange and purple was chosen as a standard, and the chromosomes in the hybrids between this and various other strains have been examined. As a rule, orange purple females have been crossed to males from the strains to be tested. The presence of the recessive mutant genes has served as a check against the possible non-virginity of the mothers. Tests of the race B strains were usually performed by crossing them to a race B strain from Klamath, California, homozygous for orange. The Klamath strain of race B differs in the gene arrangement from the standard race A strain, but the nature of the difference being known, the results obtained may be translated in terms of the standard race A arrangement.

The known distribution area of *pseudoobscura* extends from the Pacific coast to the Rocky Mountains, the western edge of the prairies, into central Texas, and from central British Columbia to southern Mexico. In

recent years a fairly large collection of strains of this species has been accumulated in this laboratory. The wild ancestors of these strains have been collected out of doors with the aid of traps containing fermenting banana mash. Both females and males come to the traps; a majority of the females are already fertilized by one or more males before coming to the traps. Each laboratory strain is derived from a single wild female. In most localities where collecting has been done several strains have been isolated. The strains are designated by the name of the locality in which their wild ancestors have been collected, and by serial numbers. Thus, "Taos-4" is the strain No. 4 from Taos, New Mexico.

Examination of the chromosomes in the hybrids between the standard strain and strains coming from various geographical localities furnishes qualitative, as well as roughly quantitative, information regarding the kinds of gene arrangements encountered in the populations inhabiting these localities. For a more precise quantitative analysis of wild populations this technique is however unsatisfactory, since the variety of gene arrangements originally present in a given strain may be decreased if this strain is kept in the laboratory for many generations. Therefore, the relative frequencies of the different gene arrangements in some localities have been determined by a different method. Wild males collected out of doors, or single sons of wild females, are outcrossed to the standard orange purple females, and the chromosome configurations are studied in several hybrid larvae from each cross. If the wild fly was a structural heterozygote (for example, if it had one third chromosome differing from the other in gene arrangement), at least two types of hybrid larvae must appear. This has been observed in many instances. Sometimes all hybrid larvae examined are alike, which may be due either to the parents being structurally homozygous, or to the smallness of the sample tested. The following convention was therefore adopted: if three or more larvae from a given cross have been examined and found to be alike, the tested individual is regarded as having been structurally homozygous; if less than three larvae have been examined and only one type was found, only one chromosome is assumed to have been tested. It may be noted that in most cases six or seven larvae were examined from each cross.

Cytological examination has been made mostly on temporary acetocarmine mounts. This method is unrivalled as a time-saving device, and

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#### EXPLANATION OF PLATE 1

Plate 1.—Above—a map of the standard gene arrangement in the third chromosome of *Drosophila pseudoobscura*, showing the division into the sections and sub-sections, and also the loci of the breakages in some of the inversions recorded in this chromosome. Below—configurations observed in the third chromosome in various inversion heterozygotes. The scales (40 and 50 micra) indicate the magnification used for making the drawings of the standard map and of the other configurations respectively.

moreover fresh temporary mounts are frequently superior even to the best permanent preparations. The drawings of the chromosomes reproduced below were made with the aid of a camera lucida, under the magnification of  $90\times$  objective,  $10\times$  ocular (Zeiss). The map of the standard third chromosome (Plate 1) is a composite drawing made up of several separate ones representing the given part of the chromosome most clearly. The magnification here is  $120\times$  objective,  $10\times$  ocular. In all the drawings which are not composites the parts of the chromosomes in which the disc patterns were not clear enough in a given preparation are represented by dotted outlines only. In general, cells in which the amount of pairing of homologous chromosome sections was at its maximum were selected for making the drawings.

#### GEOMETRICAL CONSEQUENCES OF MULTIPLE INVERSIONS

The occurrence of an inversion in a chromosome leads to the emergence of a "new" chromosome having a modified gene arrangement. This "new" chromosome may, in turn, undergo further change due to another inversion, or due to a translocation, deficiency, or to some other chromosome aberration. The occurrence subsequently of several inversions in the same chromosome may, theoretically, lead to particularly interesting results, which should be considered before the data on the gene arrangement in the chromosomes of *D. pseudoobscura* are presented.

Assume that a chromosome has genes arranged in the order ABCDEF. An inversion of the section containing the genes from B to E inclusive gives rise to a chromosome AEDCBF. If the original and the derived chromosome are present in the same individual (inversion heterozygote), a configuration resembling that shown in the upper right corner of figure 1 will be formed in the salivary gland cells.

A chromosome once changed by an inversion may undergo a further change due to another inversion. The location of the second inversion with respect to the first may vary. Three possibilities present themselves. (1) The second inversion may occur in the part of the chromosome not affected by the first. A chromosome ABCDEFGHI may be changed first to AEDCBFGHI and then to AEDCBFHGI. An individual heterozygous for ABCDEFGHI and AEDCBFHGI will have in its salivary glands a chromosome forming a double loop shown in figure 1, second line from above. Such inversions may be termed independent. (2) An inversion may take place wholly inside of the region affected by another inversion, or it may include that region. The chromosome ABCDEF changes first to AEDCBF and then to AECDBF, or else the change is  $ABCDEF \rightarrow ABDCEF \rightarrow AECDBF$ . Such inversions may be called included ones. An individual having chromosomes ABCDEF and AECDBF is expected to

have in its salivary gland cells a double loop configuration shown in figure 1, second line from below. (3) The second inversion may have one end inside and the other outside of the region included in the first inversion. For example, the chromosome ABCDEFGHI may change consecutively to AFEDCBGHI and to AFEHGBCDI. Such inversions may be described as overlapping ones. The chromosome configuration expected to appear in the salivary gland cells of an individual heterozygous for overlapping inversions is like that shown in figure 1, lower right corner.

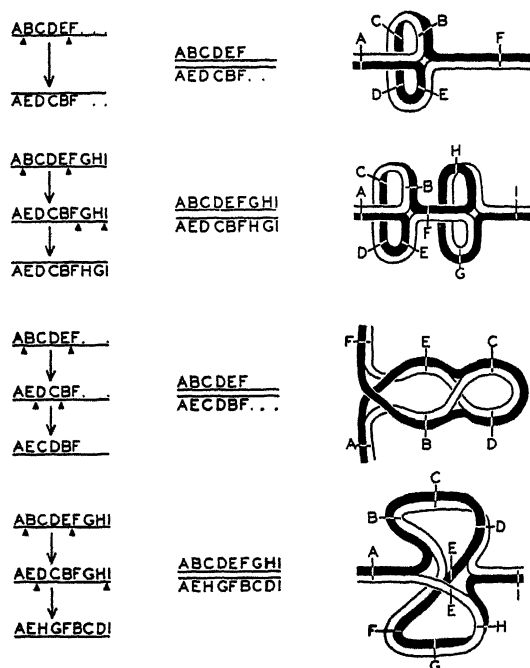


FIGURE 1.—A schematic representation of the pairing of chromosomes differing in a single or a double inversion. Above—a single inversion; second from above—two independent inversions; third from above—two included inversions; below—two overlapping inversions. Further explanation in text.

Overlapping inversions deserve an especially careful consideration. Suppose that three gene arrangements, ABCDEFGHI, AFEDCBGHI, and AFEHGBCDI, are encountered in a species. The first of these arrangements might have originated from the second through a single inversion; or else, the first might have given rise to the second, likewise through a single inversion. Similar relations exist between the second and the third of these arrangements: each of them might have originated from or given rise to the other by means of a single inversion step. But the supposition that the first has originated from the third, or vice versa, leads to great difficulties, which can be obviated only by supposing that the change has

been accomplished in two steps, the second arrangement being the intermediate stage. Indeed, in order that the chromosome ABCDEFGHI may become transformed at once into AFEHGBCDI, or vice versa, the chromosome must become broken simultaneously in four places: between A and B, D and E, F and G, and H and I. The occurrence of such a multiple breakage is in itself not very difficult to visualize, since such complex breakages have in fact been observed in some X-ray experiments with *Drosophila*. Whether they occur also without X-ray treatments is for the time being an open question. Much more important is the fact that the fragments of a chromosome broken in four places may reunite in a variety of ways: AGHEFBCDI, AEFBCDHGI, AEFHGBCDI, AFEHGBCDI, and others, all of which are theoretically equally likely to occur.

If the three arrangements, ABCDEFGHI, AFEDCBGHI, and AFEHGBCDI, are all observed to occur in nature, the probability of the direct origin of the first from the third, or vice versa, becomes almost nil. Indeed, this would involve the assumption that due to a mere coincidence the chromosome has been broken at exactly the same two places on at least two separate occasions. Since a chromosome of a moderate length may undergo breakage presumably at hundreds or even at thousands of points, such coincidences must be extremely rare. Even if the supposition is made that certain points in the chromosome are more likely to break than others, the coincidence of two breaks at exactly the same loci remains improbable, for at least some chromosomes are known to have undergone breakage at many points. It follows, then, that the phylogenetic relationships of the three gene arrangements represented above must be  $1 \rightarrow 2 \rightarrow 3$ , or  $3 \rightarrow 2 \rightarrow 1$ , or  $1 \leftarrow 2 \rightarrow 3$ , but not  $1 \rightleftharpoons 3$ . In other words, although we can not determine directly which of the three arrangements is the ancestral and which are the derived ones, if any one is selected as the original then the course of the evolution is thereby fixed.

If only the first and the third arrangements are actually observed to occur in nature, then the second may be postulated theoretically as a form that either has lived in the past but has become extinct, or else is still living but has not been discovered. For if the change from the first to the third arrangement has taken place in two steps, each involving a single inversion, there could have been but a single intermediate, namely the second arrangement. Among the seventeen gene arrangements that are known in the third chromosome of *pseudoobscura*, at least one arrangement has been so predicted, and subsequently discovered to occur on Santa Cruz Island, off the coast of California, and in some other places.

The geometrical properties of the overlapping inversions that render them amenable to a phylogenetic analysis of the sort just outlined are not



found in the independent and the included ones. If only two sequences, ABCDEFGHI and AEDCBFGHI, are encountered, their genetic relationships are ambiguous. Supposing that inversions occur one at a time, the relationships  $ABCDEF\overline{GHI} \rightleftharpoons AEDCB\overline{FGHI} \rightleftharpoons AEDCB\overline{FHGI}$  and  $ABCDEF\overline{GHI} \rightleftharpoons ABCDEF\overline{HGI} \rightleftharpoons AEDCB\overline{FHGI}$  are equally probable. In other words, the intermediate steps in the phylogenetic series can not be reconstructed with a sufficient degree of assurance, while such a reconstruction is practicable for overlapping inversions. If one of the two theoretically possible intermediates (that is, AEDCBFGHI or ABCDEFHGI) is actually encountered in nature, it is of course likely that it, rather than its alternative, is the actual connecting link between the two end members of the series. But even in this most favorable case the method remains less precise than it is for overlapping inversions; it is possible, for example, that the arrangement ABCDEFGHI has given rise independently to AEDCBFGHI and ABCDEFHGI, and that a crossing-over between the latter has produced AEDCBFHGI.

In practice, it becomes important to establish whether two or more inversions observed in the same chromosome do or do not belong to the class of the overlapping ones. Fortunately, the salivary gland chromosome method is sufficiently accurate to permit a decision to be made in every adequately studied case. Even if the degree of the overlapping is small (that is, if one of the breaks in one of the inversions lies very close to a breakage point in the other), a careful investigation shows where in the "new" chromosome every section of the "old" one is located.

#### GENE ARRANGEMENTS IN THE THIRD CHROMOSOME OF RACE A

A composite map of the standard gene arrangement in the third chromosome of race A of *pseudoobscura* is represented in the upper part of Plate 1. It should be reiterated that the choice of this arrangement as a standard is arbitrary, and the results of the investigation would not be altered in the least if a different choice were made. TAN (1935) and DOBZHANSKY and TAN (1936) have already published maps of the same standard arrangement, but it is believed that the map here given is more accurate. We do not claim however that even this map represents all the faintest discs that may be found in this chromosome.

To facilitate description, the chromosomes of *pseudoobscura* have been subdivided into one hundred arbitrary sections; the third chromosome contains sections from 63 to 81 inclusive (DOBZHANSKY and TAN 1936). Each section is further subdivided into sub-sections denoted by letters A, B, C, etc. The limits of the sections and sub-sections are indicated by lines in Plate 1. In the standard arrangements the sections run in the natural order, from the proximal to the distal end, thus: 63 ABCDE, 64

ABC, 65 ABCD . . . 81 ABCD. Gene arrangements other than the standard one can be described most conveniently in terms of these numbered sections and sub-sections.

When the strain Arrowhead-5, derived from a single female collected near the village of Arrowhead, British Columbia, is crossed to the standard, the third chromosomes in the hybrid form a pairing configuration like that represented in Plate 1. From this configuration one can easily deduce that the Arrowhead strain differs from the standard in having a single inversion in the third chromosome. The origin of this inversion can be represented as being due to a breakage of the standard third chromosome in the sections 70B and 76B, and a rotation of the middle fragment by 180°. The arrangement of sections in the third chromosome of the Standard and the Arrowhead strains are as follows:

Standard 63,64 . . . 69,70A:70BCD,71 . . . 75,76AB:76C,77 . . . 81

Arrowhead 63,64 . . . 69,70A:76BA,75 . . . 71,70DCB:76C,77 . . . 81

The signs : indicate the loci of the breakages. Configurations similar to that observed in the Arrowhead/Standard hybrids have been found also in hybrids between the standard and many other strains. It follows that the gene arrangement first detected in the Arrowhead-5 strain is encountered rather commonly elsewhere; this will be referred to as the "Arrowhead" arrangement. In strains that are homozygous for the third chromosome of the Arrowhead type no loop-like configuration similar to that shown in Plate 1 is, of course, formed. The examination of the disc pattern in the third chromosome shows however that the seriation of the sections is like that just indicated for the Arrowhead rather than that for the Standard chromosome.

In crosses between certain strains collected on the slopes of Pikes Peak Colorado, and the standard strain, the third chromosome in the salivary gland cells has been observed to form a configuration like that shown in Plate 1 under the label "Pikes Peak/Standard." Although this configuration is identical in type with that in Arrowhead/Standard hybrids, a more careful inspection shows that different sections of the chromosome are involved in the formation of the loops in the two cases. It follows that the gene arrangement found in the Pikes Peak strains is different both from that found in the Arrowhead and in the standard strains. A comparison of the standard and the Pikes Peak arrangements may be represented thus:

Standard 63,64,65AB:65CD,66 . . . 75ABC:76 . . . 81

Pikes Peak 63,64,65AB:75CBA . . . 66,65DC:76ABC . . . 81

It is worth while to compare the Arrowhead and Pikes Peak arrangements with each other. Both can be thought of as being derived from, or giving rise to, the standard arrangement by a single inversion. The two

inversions are however of the overlapping type: the distal break in Pikes Peak lies further from the free end of the chromosome than the distal break in Arrowhead, while the proximal break in Pikes Peak is much closer to the base of the chromosome than the proximal break in Arrowhead (Plate 1). The origin of the Pikes Peak arrangement directly from Arrowhead, or vice versa, is therefore very improbable. The relationships of the three arrangements may be represented as Arrowhead $\rightleftharpoons$ Standard $\rightleftharpoons$ Pikes Peak, but not as Arrowhead $\rightleftharpoons$ Pikes Peak. In hybrids carrying one Arrowhead and one Pikes Peak chromosome a configuration resembling the double loop shown in figure 1, lower right corner, may be expected. One of these loops must be formed by the sections from 65C to 70A inclusive (corresponding to FGH in figure 1), and the other loop by sections from 70B 75C (corresponding to BCD in fig. 1), while the base (sections 63 to 65B) and the free end (sections 76B to 81) must pair normally (corresponding to I and A in fig. 1). Sections 76A' and 76B are too short to be expected to pair frequently with their homologues (corresponding to E in fig. 1). The cross Arrowhead by Pikes Peak has actually been made, and the expected chromosome configuration has been observed in the hybrids.

Crosses between standard and certain strains from Santa Cruz Island California, show in the third chromosome a double-loop configuration depicted in Plate 1. An analysis of this configuration leads to the conclusion that the arrangement of sections in the third chromosome of the Santa Cruz strains is as follows:

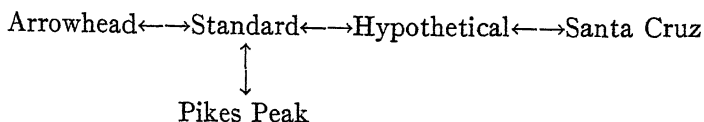
63 . . . 67,68ABC:79BCD:76A,75 . . . 69,68D:79A,78,77,76CB:80,81

It is interesting to determine in what relation the Santa Cruz sequence stands to the previously described ones. An attempt to derive the Santa Cruz sequence from either Arrowhead or Pikes Peak shows immediately that at least three inversion steps are necessary, one of which transforms the Arrowhead or Pikes Peak sequences respectively into the standard one. Only two inversion steps are needed to derive Santa Cruz from the standard arrangement. These two steps can be represented as follows:

Standard 63 . . . 67,68ABCD,69 . . . 75,76A:76BC,77,78,79ABCD:80,81  
Hypothetical 63 . . . 67,68ABC:D,69 . . . 75,76A,79DCB:A,78,77,76CB,  
80,81

Santa Cruz 63 . . . 67,68ABC,79BCD,76A,75 . . . 69,68D,79A,78,77,  
76CB,80,81

The hypothetical link between the standard and Santa Cruz has as yet not been discovered in *pseudoobscura*. It will be shown below that the essential characteristics of the hypothetical sequence are encountered in the X<sup>2</sup> chromosome of a related species, *miranda*. The relationships of the sequences described thus far are therefore as follows:



Crosses between the standard strain and certain strains derived from flies collected at Cuernavaca, Morelos, Mexico, showed in the third chromosome a configuration reproduced in Plate 1. This configuration, though at first sight very complex, can be resolved to indicate the following arrangement of sections in the chromosome of the Cuernavaca strain:

63,64AB,69DE,70 . . . 75,76A,79DCB,68CBA,67 . . . 64C,69CBA,68D,79A,78,77,76CB,80,81

The following series of steps was worked out theoretically to account for the origin of the Cuernavaca sequence (IV) from the standard one (I):

(I) 63,64ABC,65 . . . 67,68ABCD,69ABCDE,70 . . . 75,76A:BC,77,78,79ABCD:80,81

(II) 63,64ABC,65 . . . 67,68ABC:D,69ABCDE,70 . . . 75,76A,79DCB:A78,77,76CB,80,81

(III) 63,64AB:C,65 . . . 67,68,ABC79BCD,76A,75 . . . 70,69ED:CBA,68D,79A,78,77,76CB,80,81

(IV) 63,64AB,69DE,70 . . . 75,76A,79DCB,68CBA,67 . . . 65,64C,69CBA,68D,79A,78,77,76,CB,80,81

It may be noted that six breakages have to be assumed in the chromosome to derive the Cuernavaca sequence from the standard one; it would require eight breakages to derive Cuernavaca from either Arrowhead or Pikes Peak. The arrangements (II) and (III) were provisionally designated as "hypothetical 1" and "hypothetical 2" respectively. Shortly thereafter, due to accession of material from Santa Cruz Island, the Santa Cruz arrangement was discovered, and proved to be identical with the theoretically postulated "hypothetical 2" (= III). To test this identification, a simple experiment was made, namely a Cuernavaca strain was crossed to a Santa Cruz strain. If the Santa Cruz and the "hypothetical 2" arrangements are identical, a single inversion is expected in the hybrid, the sections from 64C to 69ED inclusive being in the inversion loop. The observations have borne out this prediction. Hence, no more than a single hypothetical arrangement need be postulated to account for the descent of the Cuernavaca arrangement from the standard one, or vice versa. The line of descent may be represented as follows:

[Standard  $\longleftrightarrow$  "hypothetical"  $\longleftrightarrow$  Santa Cruz  $\longleftrightarrow$  Cuernavaca]

Certain strains from the Chiricahua Mountains, Arizona, produce when crossed to the standard a pairing configuration in the third chromosome shown in Plate 1. An analysis of this configuration leads to the conclusion that a gene arrangement not identical with those described previously is

found in the Chiricahua strains. This new arrangement, denoted Chiricahua I, turns out to have the following sequence of sections:

63 . . . 67,68ABC,79BCD,76A,75 . . . 71,78ABC,79A,68D,69,70,77,76CB,80,81

A comparison of the Chiricahua I arrangement with the standard one shows that a triple inversion is present; six breaks in the chromosome have to be assumed to derive one from the other. The origin of the Chiricahua I becomes clearer if it is compared with the Santa Cruz arrangement, for they are identical, except in the sub-terminal portion of the chromosome. One can be derived from the other by a single inversion, as follows:

Santa Cruz 63 . . . 67,68ABC,79BCD,76A . . . 71:70,69,68D,79A,78CBA:77,76CB,80,81

Chiricahua I 63 . . . 67,68ABC,79BCD,76A . . . 71:78ABC,79A,68D,69,70:77,76CB,80,81

The cross Chiricahua I  $\times$  Santa Cruz gives the expected configuration, namely the single inversion loop shown in Plate 2. Chiricahua I can not be derived from Cuernavaca, or vice versa, by a single inversion, since they are related as overlapping inversions. They can be derived from each other only through the intermediate step of the Santa Cruz arrangement, as follows:

Chiricahua I  $\longleftrightarrow$  Santa Cruz  $\longleftrightarrow$  Cuernavaca

Three separate strains from Mammoth Lake, Sierra Nevada, California, produced when crossed to the standard a configuration not encountered in any other crosses. Unfortunately, these three strains were lost before this new arrangement, Mammoth, was studied in detail and before appropriate drawings could be made. The most probable (though not conclusively established) sequence of sections in the Mammoth chromosome appears to be as follows:

63 . . . 67,68ABC,79BCD,76A:76BC,77,78,79A,68D,69 . . . 75:80,81

If this interpretation is correct, the Mammoth arrangement can be derived from Santa Cruz by a single inversion. No test of this interpretation by crossing the two to each other has been made however.

Still another, and very interesting, arrangement has been detected in a strain coming from the tree line region of Pikes Peak, Colorado (elevation more than 11,000 feet). The heterozygote Tree Line/Standard shows a configuration in the third chromosome represented in Plate 1. The sequence of sections in the Tree Line chromosome, compared to that in Santa Cruz, is as follows:

Santa Cruz 63 . . . 67,68ABC,79BCD,76A,75,74C:74BA,73 . . . 69:68D,79A,78,77,76CB,80,81

Tree Line 63 . . . 67,68ABC,79BCD,76A,75,74C:69 . . . 73,74AB:68D,79A,78,77,76CB,80,81

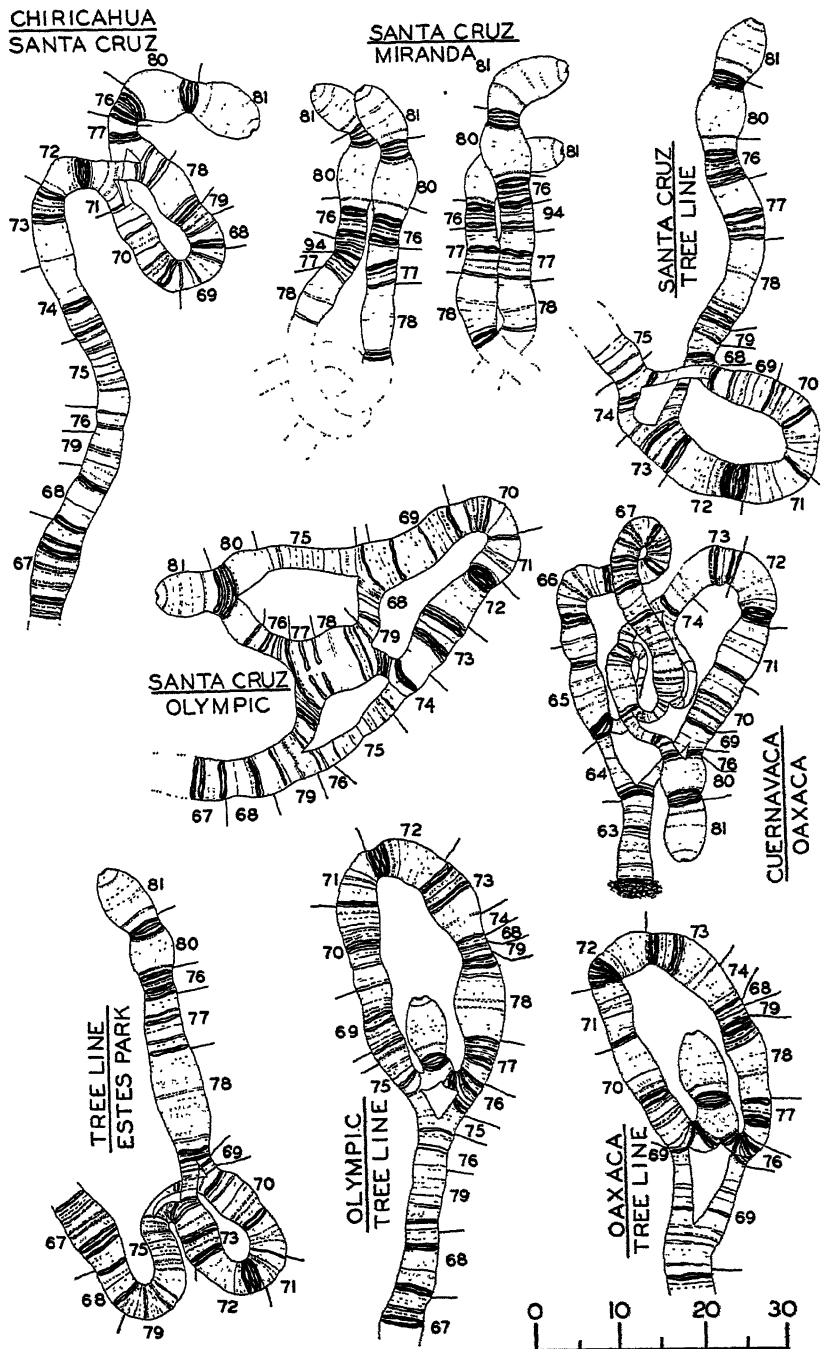


Plate 2.—Configurations observed in the third chromosome in various inversion heterozygotes. The scale below represents 30 micra.

The Tree Line arrangement may, consequently, be derived from Santa Cruz, or vice versa, by a single inversion. The derivation of Tree Line from any other arrangement described above demands more inversion steps, namely, two (Cuernavaca, Chiricahua I, Mammoth), three (Standard), or four (Arrowhead, Pikes Peak). In every case an arrangement identical with Santa Cruz has to be postulated if the origin is to be accomplished with a minimum of inversion steps. The heterozygote Tree Line/Santa Cruz shows, as expected, a single fairly short inversion in the third chromosome (Plate 2).

In turn, the Tree Line arrangement is very simply related to three other chromosome arrangements, each of which can be derived from Tree Line, and from no other arrangement, through a single inversion step. These three new arrangements were first recovered from strains coming respectively from Estes Park, Colorado, from mountains near Brinnon, Olympic peninsula, Washington, and from Cerro San Jose, Oaxaca, Mexico. They were designated as the Estes Park, Olympic, and Oaxaca arrangements respectively. A comparison of the Tree Line arrangement with the three new ones is shown below:

Tree Line 63 . . . 67,68ABC,79BCD,76A,75C:BA,74C,69ABC:DE,70 . . .

73,74AB,68D,79A:78,77,76C:B,80A:BC,81

Estes Park 63 . . . 67,68ABC,79BCD,76A,75CBA,74C,69ABC:79A,68D,

74BA,73 . . . 70,69ED:78,77,76,CB,80ABC,81

Olympic 63 . . . 67,68ABC,79BCD,76A,75C:80A,76BC,77,78,79A,68D,

74BA,73 . . . 70,69EDCBA,74C,75AB:80BC,81

Oaxaca 63 . . . 67,68ABC,79BCD,76A,75CBA,74C,69ABC:76C,77,78,

79A,68D,74BA,73 . . . 70,69ED:76B,80ABC,81

To test the above interpretation, the crosses Estes Park  $\times$  Tree Line, Olympic  $\times$  Tree Line, and Oaxaca  $\times$  Tree Line were made. As expected, in each of the crosses the third chromosome showed a single inversion, but the sections included in the inversion loop were different in each case (Plate 2). All three inversions belong to the overlapping class, although the proximal breaks in Estes Park and Oaxaca are very close to each other, being located at approximately the middle of the length of section 69. The relationships of the four arrangements under consideration may be represented as follows:



The crosses Olympic  $\times$  Santa Cruz (Plate 2), Olympic  $\times$  Estes Park, and Oaxaca  $\times$  Estes Park were also made. The third chromosomes in the hybrids showed double loop configurations with some unpaired sections, as

expected in overlapping inversions. The crosses Estes Park×Standard (Plate 1), Olympic×Standard (Plate 1), Oaxaca×Standard, Oaxaca×Cuernavaca (Plate 2), Oaxaca×Chiricahua I, Estes Park×Chiricahua I, and Oaxaca×Pikes Peak give very complex pairing configurations in which many chromosome sections usually fail to pair with their homologues. All these relationships are in accord with expectations, since Estes Park, Olympic, Oaxaca, and Santa Cruz can be derived from each other through two inversion steps, the Tree Line arrangement being in every case the connecting link which could be constructed theoretically if it were not actually observed to occur in nature. It takes however three steps to derive any of these arrangements, except Santa Cruz, from either Chiricahua I or Cuernavaca, and four steps to derive them from the standard arrangement.

In addition to the eleven arrangements thus far described, one more, the twelfth, has been observed in race A. In a cross between a strain homozygous for the Arrowhead arrangement and Chiricahua-6, a larva was found that exhibited a configuration of an unusual type. The distal part of the third chromosome had a sequence of sections identical with that encountered in Arrowhead, but there was a short inversion in the middle part, involving approximately sections 69, part of 70, part of 76, and 75. Obviously, some of the flies in the Chiricahua-6 strain had a gene arrangement not hitherto encountered; this arrangement is labeled Chiricahua II. Attempts to recover the Chiricahua II sequence from the strain in which it was once observed proved unavailing, and this sequence has never been seen again. Chiricahua II is clearly derived from the Arrowhead arrangement through a single inversion, its relationship to other arrangements being as follows:

Standard←→Arrowhead←→Chiricahua II

#### GENE ARRANGEMENTS IN THE THIRD CHROMOSOME OF RACE B

The gene arrangement in the third chromosome of race B is apparently about as variable as it is in race A. Relatively few strains of race B have been examined however, and therefore the number of arrangements recorded in this race is smaller than in race A, namely six. TAN (1935) has shown that the standard arrangement is encountered in race B as well as in race A, but he has also found that race B usually has another gene sequence in the third chromosome, giving a single inversion in the third chromosome in interracial hybrids with Standard race A. This most frequent arrangement in race B is denoted as Klamath, according to the name of the locality (in northern California) from which one of the strains carrying it was collected.

The hybrids between race B strains possessing a third chromosome of



the Klamath type, and either race A or race B strains with the standard arrangement, show an inversion loop depicted in Plate 1. This inversion occupies approximately the same part of the chromosome as that found in Standard  $\times$  Arrowhead heterozygotes (Plate 1), but a careful examination discloses that the two inversions are unquestionably not identical. The sequence of sections in the Klamath chromosome is as follows, the Arrowhead sequence being given for comparison:

Klamath 63 . . . 69, 70ABCD:77,76CBA,75 . . . 71:78,79,80,81

Arrowhead 63 . . . 69, 70A:76BA,75 . . . 71,70DCB:76C,77,78,79,80,81

In the Klamath B  $\times$  Arrowhead A hybrids no inversion loop is present in the third chromosome, but two sections, one lying at about the middle and the other in the distal part of the chromosome, remain unpaired. These are sections 70BCD, 76C, and 77. Klamath may be derived from Standard, or vice versa, through a single inversion, but it takes two steps to pass from Arrowhead to Klamath. The relationships of the three sequences are therefore as follows:

Klamath  $\longleftrightarrow$  Standard  $\longleftrightarrow$  Arrowhead

Three strains of race B from Sequoia National Park, California, were crossed to a strain possessing the Klamath chromosome. The hybrids showed in the third chromosome a double inversion (fig. 2). The gene arrangement encountered in these three strains is referred to as Sequoia I; it is characterized by the following sequence of sections:

63 . . . 69,70:73,72,71:74,75 . . . 80,81

It may be noted at once that the Sequoia I arrangement may be derived through a single inversion from the standard, but it takes two steps to obtain Sequoia I from Klamath, or vice versa. Nevertheless, we are not dealing here with overlapping inversions, since the proximal breakages in Sequoia I and Klamath seem to lie at the same point, corresponding to the dividing line between sections 70 and 71. The Sequoia I inversion may be described as being included in the Klamath one, with one end apparently coinciding. Hence, the relationships between Sequoia I and Klamath may be pictured in the following two ways. First: 63 . . . 70:77 . . . 74:73 . . . 71:78 . . . 81 (Klamath)  $\longleftrightarrow$  63 . . . 70:71 . . . 73:74 . . . 77:78 . . . 81 (Standard)  $\longleftrightarrow$  63 . . . 70:73 . . . 71:74 . . . 77:78 . . . 81 (Sequoia I), or, second, 63 . . . 70:77 . . . 74:73 . . . 71:78 . . . 81 (Klamath)  $\longleftrightarrow$  63 . . . 70:77 . . . 74:71 . . . 73:78 . . . 81 (hypothetical)  $\longleftrightarrow$  63 . . . 70:73 . . . 71:74 . . . 77:78 . . . 81 (Sequoia I). The second of these two manners of representation demands a hypothetical connecting link, while the first does not. Since the standard arrangement is encountered in race B populations inhabiting the region of the Sequoia National Park, by far the most probable course of evolution is as follows:

Sequoia I  $\longleftrightarrow$  Standard  $\longleftrightarrow$  Klamath,

but it should nevertheless be remembered that the degree of probability with which this relationship is established is lower than that involved in other conclusions presented above.

Three other gene arrangements have been detected in strains of race B coming from, respectively, Cowichan Lake, British Columbia, the neighborhood of Wawona, California, and from Sequoia National Park, California. These arrangements are denoted as Cowichan, Wawona, and

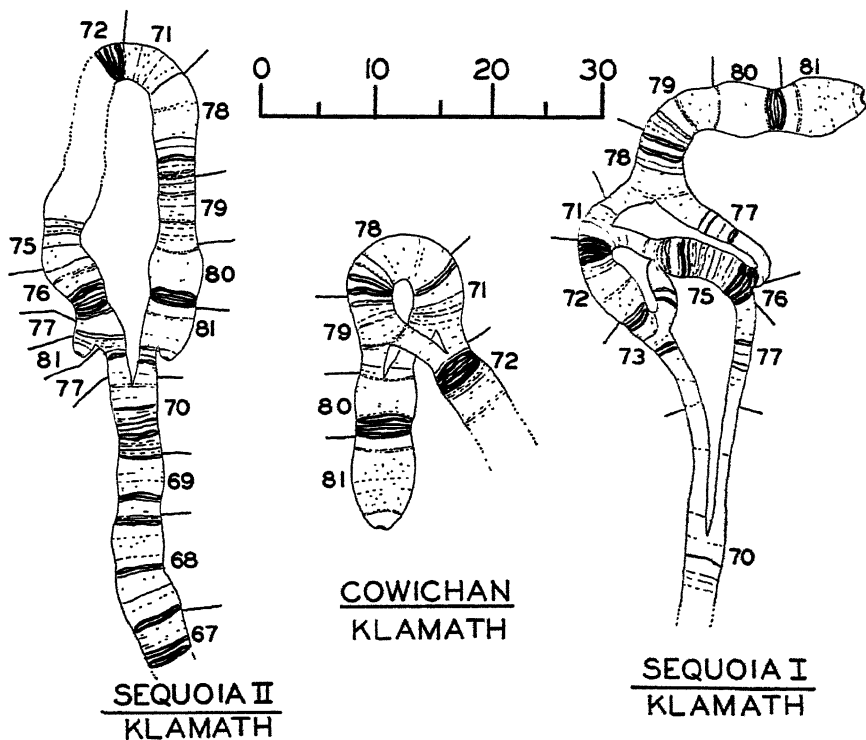


FIGURE 2.—Configurations in the third chromosome in hybrids between various strains of race B.

Sequoia II. The pairing configurations encountered in the hybrids Sequoia II  $\times$  Klamath and Cowichan  $\times$  Klamath are shown in fig. 2, and that in the hybrid Wawona  $\times$  Standard in Plate 1. The distribution of sections in the three new arrangements is as follows (the data for Klamath being given for comparison):

Klamath 63 . . . 70, 77B:A, 76:75 . . . 71C:BA, 78A:BC, 79ABC:D, 80, 81AB:CD

Cowichan 63 . . . 70, 77BA, 76, 75 . . . 71C:79CBA, 78CBA, 71AB:79D, 80, 81ABCD

Wawona 63 . . . 70, 77BA, 76:78A, 71ABC . . . 75:78BC, 79ABCD, 80, 81ABCD

Sequoia II 63 . . . 70, 77B:81BA, 80, 79DCBA, 78CBA, 71ABC . . . 75, 76, 77A:81CD

Each of the three new arrangements can be derived from Klamath by a single inversion, while it takes two inversions to derive them from the Standard or from each other. The Cowichan and Wawona inversions are overlapping, and therefore the relationship

Cowichan  $\longleftrightarrow$  Klamath  $\longleftrightarrow$  Wawona

may be taken as established. The inversion encountered in Sequoia II is much longer than that found either in Cowichan or in Wawona, and includes the regions involved in the other two. The relationships between these arrangements are therefore similar to those discussed above in connection with the series Klamath-Standard-Sequoia I. In other words, since the Klamath arrangement is known to be present in nature, the most probable inference is that Sequoia II has descended directly from Klamath, or has given rise to the latter, independently from the origin of Wawona and Cowichan. If however, the Klamath arrangement were unknown, one could formulate two equally probable hypotheses, first, that the ancestral arrangement was like Klamath, or, second, that the ancestral arrangement was one combining the properties of Sequoia II and Wawona, or of Sequoia II and Cowichan. Such hypothetical arrangements might have given rise to the existing ones through single inversions.

On the other hand, the relationships between Sequoia II, Klamath, and Standard are quite clear. The inversions differentiating these arrangements are all overlapping, and therefore Sequoia II can not arise directly from Standard, or vice versa. The same is true for Wawona and Cowichan. Hence, the relationships are as follows:

Sequoia II  $\longleftrightarrow$  Klamath  $\longleftrightarrow$  Standard

Wawona  $\longleftrightarrow$  Klamath  $\longleftrightarrow$  Standard

Cowichan  $\longleftrightarrow$  Klamath  $\longleftrightarrow$  Standard

Taken as a whole, the six gene arrangements recorded in race B are not related to any of the arrangements known in race A, except through the Standard one. Since, however, the Standard is the only arrangement encountered in race A as well as in race B, this connecting link is sufficient to account for the origin of all arrangements in both races from a common source.

#### THE EFFECTS OF INVERSIONS IN THE THIRD CHROMOSOME ON CROSSING OVER

*Standard sequence.* The map of the third chromosome given by STURTEVANT and TAN (1937) is based on the results obtained from females homozygous for the standard sequence. The following loci are included; orange

(o), Blade (7.7), abrupt (9.7), Emarginate (13.3), Jagged (23.3), Scute and polychaete (28.3), purple (49.9), curved ( $65 \pm$ ), crossveinless (68.0). So far as tested, values similar to these are obtained when one of the "Standard" chromosomes is derived from race B, though the usual complications (differential viability and effects of inversions in other chromosomes) are evident here as in all crossing over experiments with A  $\times$  B hybrids.

*Arrowhead sequence.* In females of the constitution Standard/Arrowhead the orange-Scute interval gives about 8 percent crossing over (192 out of 2511, = 7.6 percent in one series), but the results appear to be variable. Orange, Blade, and Emarginate have all given crossing over with Scute; Scute and the inversion have never given crossing over. One doubtful case suggests a crossover between the inversion and purple, and a more certain one indicates that crossveinless lies to the right of the inversion. The total frequency of Scute-crossveinless crossing over is certainly very low, not more than 0.1 percent.

Several mutant genes have been found in Arrowhead chromosomes: a Jagged allelomorph, plexus, narrow, and rugose. We have not succeeded in getting crossing over between any of these and the inversion in Standard/Arrowhead. In homozygous Arrowhead the data indicate the following map: orange (o), Emarginate (14.1), plexus (22.1), Jagged (23.1), narrow (25.1), rugose (29.7). Taking these data, together with the results of TAN (1937), it may be concluded that Jagged and crossveinless are not in the inverted section of Arrowhead, Scute and rugose are in it, and purple and narrow are doubtful.

*Pikes Peak.* Standard/Pikes Peak has given crossing over between purple and crossveinless, a Pikes Peak crossveinless chromosome being easily obtained. No crossovers have been observed elsewhere. An experiment involving orange, Blade, Scute, and purple was carried out on the assumption that so long an inversion should give recoverable double crossovers, but none was obtained.

*Klamath.* In Standard-B/Klamath the results (orange Scute purple tested) are much like those from Standard-A/Arrowhead, plus the usual A  $\times$  B complications. Orange-Scute crossovers are present in nearly every culture, Scute-purple ones have never been found.

*Other sequences.* No systematic study has been carried out for the remaining sequences, but a few data have been obtained in experiments using orange Scute purple (standard) against some of them. In such experiments Scute-purple crossovers have never been found; orange-Scute ones occur rarely (approximately 1 percent) when the opposing chromosome is Chiricahua, Estes Park, or Oaxaca. They have not been found in the cases of Santa Cruz, Cuernavaca, or Tree Line, but the numbers of flies seen are too low to be significant. Comparison of the cytological maps

(TAN, in press) with the limits of the inversions here given indicates that Pikes Peak and Cuernavaca would be expected not to give single crossovers between orange and Scute; Santa Cruz and Tree Line may be expected to produce them as frequently as do Chiricahua, Estes Park, and Oaxaca—a conclusion not negated by the small amount of data available.

*Combinations not involving standard.* A few experiments have been carried out with Arrowhead/Pikes Peak and Arrowhead/Klamath, since these combinations might be expected to give single crossovers in the common inverted region (STURTEVANT and BEADLE 1936). None was found, and the probability is high that, if they occur, the crossover chromosomes are inviable. Such a result is not unexpected, since inversions as different as these in the X chromosome of *melanogaster* often give no viable crossovers. In the case of Arrowhead/Klamath, where the inversions are more alike, the present data are not conclusive, owing to the scarcity of usable mutant genes. The experiment is also complicated by the fact that the Klamath chromosome used was (of necessity) originally derived from race B, and the flies carrying it were not fully viable and fertile.

*Disturbances in chromosome pairing.* In connection with the data on the reduction of the frequency of crossing over in inversion heterozygotes, it has been found that the pairing of the chromosomes differing in gene arrangement is frequently disturbed in the salivary gland cells. As pointed out above, most of the drawings in Plates 1 and 2 and in figure 2 are made from selected figures showing as complete a pairing as can be found. In general, failures of pairing are rather common. Where only a single inversion is present (Standard/Arrowhead, Standard/Pikes Peak, Standard/Klamath, and others) the pairing is usually, though by no means always, nearly complete, only short sections immediately adjacent to the breakage points failing to pair. But where the homologous third chromosomes differ by double, triple, or multiple inversions some fairly long sections nearly always fail to pair with their homologues. In the hybrids Oaxaca/Standard, Estes Park/Standard, and many similar ones, the chromosomes usually pair only in the basal regions and in a few places in the parts affected by the inversions. Instances of cells where the third chromosomes have failed to pair almost entirely are not uncommon. No drawing of the Oaxaca/Standard configuration is given in the present article because we have not succeeded in finding a cell in which the third chromosomes of this hybrid show more than a negligible amount of pairing in the inverted region. Although small failures of pairing are sometimes encountered in structurally homozygous individuals as well, there can be no doubt that in inversion heterozygotes such failures are both more frequent and more extensive.

## GENE ARRANGEMENT IN CHROMOSOMES OTHER THAN THE THIRD

The third chromosome is by far the most variable one with respect to the gene arrangement. Nevertheless, the gene arrangement has been found to vary to some extent also in all other chromosomes, except the small fifth, which, however, has not been carefully examined in most preparations. TAN (1935) found that the left limb of the X chromosome in race A differs from that in race B in having an inverted section. As far as we know, this difference is constant, in the sense that all strains of race A differ from all race B strains in having this inversion.

Three different gene arrangements are known in the right limb of the X chromosome. Race A differs from race B in having an inversion in this limb (TAN 1935), but the so-called "sex-ratio" strains in race B have the same arrangement as the normal representatives of race A (STURTEVANT and DOBZHANSKY 1936b). "Sex-ratio" strains in race A differ from normal A by an inversion, which is however not identical with that differentiating normal race B. Cytologically, the three sequences are related as overlapping inversions:

Sex-ratio A  $\longleftrightarrow$  Normal A, or Sex-ratio B  $\longleftrightarrow$  Normal B

Six different gene arrangements have been recorded in the second chromosome, three in race A and three in race B. This chromosome carries sections from 43 to 62 inclusive, which in most race A strains are arranged in the natural order; this is the "Standard" arrangement for the second chromosome. Most strains of race B have sections inverted from the distal part of 52 to 56 inclusive (TAN 1935, DOBZHANSKY and TAN 1936). Denoting the proximal part of a section as p, and the distal part as d, the two sequences may be represented as follows:

Standard 43 . . . 51p:51d, 52 . . . 56:57 . . . 62

Race B 43 . . . 51p:56 . . . 52, 51d:57 . . . 62

Three strains of race A proved to carry a gene arrangement deviating from the Standard. These strains are Zuni-4, Magdalena-2, and Pinos Altos-9, all from New Mexico. The new arrangement, denoted as Zuni, is therefore restricted to a relatively narrow geographical region, outside of which it is not encountered. Even in New Mexico most strains have the Standard arrangement, and the three exceptional strains just enumerated were all inversion heterozygotes, carrying one Standard and one Zuni second chromosome. The Zuni arrangement differs from the Standard in having the segment extending approximately from section 47 to 53 inverted. The limits of this inversion overlap those distinguishing race B from the Standard, and consequently the relationships of the three arrangements may be represented as follows:

Zuni  $\longleftrightarrow$  Standard  $\longleftrightarrow$  race B

TAN (1935) has observed a long inversion in the second chromosome in

the hybrids between the strain Santa Lucia-7 (California) and the strain carrying the mutants Bare and Smoky. The latter is known to possess the Standard arrangement. From the published figure of this inversion it appears that the Santa Lucia arrangement is unquestionably different from both Zuni and Race B arrangements. Whether or not the Santa Lucia inversion overlaps Zuni and Race B is not clear, but in any case it takes one inversion-step to derive the Santa Lucia arrangement from the standard one, while it takes two steps to derive it from either Zuni or race B.

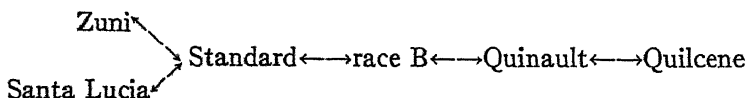
Two strains of race B have been found to deviate from the normal structure of the second chromosome in that race. Some of the larvae in the crosses cinnabar (race B)  $\times$  Quinault-23 (Olympic Peninsula, Washington) had a fairly long inversion in the distal part of the second chromosome. Some of the hybrids in the cross cinnabar  $\times$  Quilcene-4 (Olympic Peninsula) had a double inversion in the same part of the chromosome. The sequence of sections in these arrangements is as follows:

Race B 43 . . . 51p, 56d:56p, 55 . . . 52, 51d, 57:58 . . . 61, 62p:62d

Quinault 43 . . . 51p, 56d:62p, 61 . . . 58:57, 51d, 52 . . . 55, 56p:62d

Quilcene 43 . . . 51p, 56d, 62p, 61 . . . 58:56p, 55 . . . 52, 51d, 57:62d

The distal breakages in the Quilcene and Quinault inversions both lie in section 62, but it appears that in the former the exact point of the break is somewhat closer to the free end of the chromosome than in the latter. If this is the case, the two inversions are overlapping, but the material studied is not extensive enough to make this conclusion certain. In any event, the most probable relationship of the three arrangements is that Quinault is derived from the race B one, and Quilcene is derived from Quinault. The relationships of all the arrangements recorded in the second chromosome may be represented by the following scheme:



The fourth chromosome proves to be the least variable one (neglecting the small fifth). It has identical gene arrangements in race A and race B, as attested by the complete pairing of the homologues in the interracial hybrids. The only variation observed in this chromosome has been found in some larvae from the cross of the strain Cuernavaca-5 (Mexico) to a laboratory strain carrying the mutant gene plexus. The fourth chromosome in some of the hybrids showed a large inversion extending from almost the free end to the sub-basal region of the chromosome. Since the plexus strain has been repeatedly used for cytological examinations of various hybrids, and showed a normal fourth chromosome, the inversion came presumably from the Cuernavaca-5 strain.

## COMPARISON OF DROSOPHILA PSEUDOOBSCURA WITH D. MIRANDA

DOBZHANSKY and TAN (1936) have compared the disc patterns in the salivary gland chromosomes of *D. pseudoobscura* with those in a closely related species, *D. miranda*. They found that the gene arrangements in the two species are profoundly different, at least one hundred breakages being needed to derive the chromosome structure encountered in one of the species from that in the other. Yet, one may inquire to which of the gene arrangements known in *pseudoobscura* the gene arrangement of *miranda* is most closely related. Fortunately, the overlapping inversion method permits an unequivocal answer to this question.

As pointed out by DOBZHANSKY and TAN (1936), it takes one less inversion step to derive the gene arrangement in the left limb of the X chromosome in *miranda* from that in race A than from that in race B of *pseudoobscura*. Furthermore, the interracial inversion overlaps the limits of the inversion distinguishing race A and *miranda*. Hence, the relationships are as follows:

$$\text{Race B} \leftarrow \rightarrow \text{Race A} \leftarrow \rightarrow \text{miranda}$$

A comparison of the right limbs of the X chromosome and of the second chromosomes leads to conclusions fully consistent with the above: in either case the *miranda* arrangement is related to that in race A rather than to that in race B of *pseudoobscura* (inversions are overlapping). As to the third chromosome, the data of DOBZHANSKY and TAN were inconclusive. One may note here that the homologue of the third chromosome of *pseudoobscura* is present in *miranda* in duplicate in the females but only once in the males, and is termed the X<sup>2</sup> chromosome (DOBZHANSKY 1935). The proximal part of the X<sup>2</sup> contains sections 65, 66, 68, 70, and 71, and is therefore similar to the proximal part of the third chromosome of *pseudoobscura* (standard arrangement). Section 71 in *miranda* is however followed distally by a long region the homology of which was left undecided in the article of DOBZHANSKY and TAN (loc. cit.), since this region has never been observed to be paired with anything in the *miranda* × *pseudoobscura* hybrids. However, this region contains parts much resembling in disc patterns the sections 72 and 73 of *pseudoobscura*, and arranged in a similar manner (cf. drawings of DOBZHANSKY and TAN, 1936). Finally, the free end of the X<sup>2</sup> has sections 80 and 81, a condition encountered also in *pseudoobscura*. Proximally from section 80 the X<sup>2</sup> carries a series of prominent discs, the origin of which has not been determined, except that a few of them were found to be homologous to a part of section 94 in the fourth chromosome of *pseudoobscura*.

When the Santa Cruz arrangement in the third chromosome of *pseudoobscura* became known, we were struck by the resemblance of the disc pat-



terns in the distal parts of that chromosome and of the  $X^2$  chromosome of *miranda*. A cross of *pseudoobscura* Santa Cruz  $\times$  *miranda* was therefore made, and the hybrid larvae were studied cytologically. Several cells have been found in which the distal portions of the  $X^2$  and of the third chromosome were paired. Two drawings in Plate 2 show the configurations observed. An analysis of these configurations leads to the conclusion that the distal portions of these chromosomes are built as follows:

Third chromosome of Santa Cruz . . . 78, 77, 76CB, 80, 81

$X^2$  chromosome of *miranda* . . . 78, 77, 94 (part), 76CB, 80, 81

In other words, except for the insertion of a part of section 94 in *miranda*, the two chromosomes are similar in this region. Assuming, further, that the identification of sections 72 and 73 in the  $X^2$  chromosome of *miranda* is correct, we are forced to conclude that the  $X^2$  chromosome has a gene arrangement most similar to the "hypothetical" one, postulated as a connecting link between standard and Santa Cruz in *pseudoobscura* (cf. page 36). It does not follow of course that the "hypothetical" arrangement in *pseudoobscura* is identical with that observed in *miranda*. In fact, this is certainly not the case, since many differences between the two are present. Nevertheless, it may be taken for established that it takes less alteration to derive the *miranda* sequence from the "hypothetical" one in *pseudoobscura* than from any other.

Thus, all the information now available agrees in pointing to a closer similarity between race A of *pseudoobscura* and *miranda* than between race B and *miranda*.

#### PHYLOGENY OF THE GENE ARRANGEMENTS IN THE THIRD CHROMOSOME

The various separate phylogenetic schemes presented above have all been combined in one diagram in figure 3. In the earlier sections the schemes were presented with double-headed arrows, since there is no inherent method of determining the directions in which inversion phylogenies should be read. The overlapping inversion method would not prevent us from supposing that any one of the gene arrangements shown in figure 3 is the ancestral one; but once an arrangement is selected as ancestral, the course of the evolution is determined. In figure 3 most of the arrows are single-headed; it is the purpose of the present section to show how we have arrived at this result.

It is simplest to suppose that the common ancestor of *pseudoobscura* and *miranda* had the "hypothetical" sequence, since *miranda* is related to no other existing *pseudoobscura* sequence except through this one. Owing to its exceptional sex chromosome mechanism, *miranda* cannot be considered ancestral to *pseudoobscura*, but must be derived either from *pseudoobscura* itself, or from a common ancestor that was (at least as regards its X and

its third chromosomes) more like *pseudoobscura*. Further, it may reasonably be supposed that the separation took place long ago, in order to account for the great difference between *miranda* and *pseudoobscura*. The "hypothetical" sequence is thus to be considered a very ancient one.

Next to the difference between the species *pseudoobscura* and *miranda*, the most striking and important distinction in the group is that between races A and B, which have practically reached a species status, except for

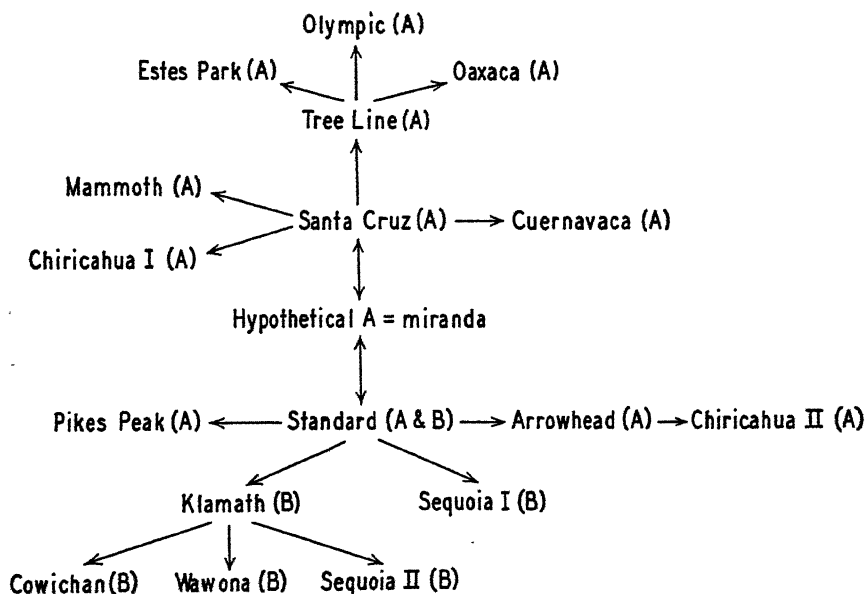


FIGURE 3.—Phylogeny of the gene arrangements in the third chromosome of *Drosophila pseudoobscura*. Any two arrangements connected by an arrow in the diagram differ by a single inversion. Further explanation in text.

the fact that they remain indistinguishable morphologically. The difference between these "races" may likewise be supposed to be of long standing. It is clear that the common ancestor of A and B had the Standard sequence, since this is the only one common to the two, and is a necessary connecting link between all other race A sequences on one hand and all other race B sequences on the other hand.

The great antiquity of the Standard and "hypothetical" sequences may thus be taken for established. There is one other arrangement that may be supposed to be relatively old, namely, Santa Cruz. Only Standard and Santa Cruz form centers to which a whole series of other arrangements are directly related (fig. 3), and only these two are derived by single steps from the "hypothetical" arrangement.

The most probable view seems to be that the original form was either

Standard or "hypothetical," with Santa Cruz still to be considered as a possibility. The argument cannot be taken as excluding other arrangements, though all those restricted to race B are highly improbable. It is, of course, entirely possible that the common ancestor of race A and race B, as well as that of *D. pseudoobscura* and *D. miranda*, was itself variable in gene arrangement, just as its living derivatives are.

#### GEOGRAPHICAL DISTRIBUTION OF THIRD CHROMOSOME GENE ARRANGEMENTS

A list of localities and strains in which the various third-chromosome gene arrangements have been recorded is given below; figures 4 and 5 present the same data in map form.

##### RACE A

*Standard.* British Columbia: Lytton-1; Merritt-2; Lake Okanagan-4, 5, 8; Lake Shuswap (Mara)-3; Kaslo-3, 4.

Washington: La Grande-2; Chelan-2, 8, 10, 12; Metaline Falls-1; The Dalles-2.

Montana: Bitterroot Mts.-6.

Oregon: Newbery Crater-3.

Northern California and Sierra Nevada: Dunsmuir-3, 9; Lassen-1, 19; Oakland-1; Pacific Grove-2; Santa Lucia-35; Wawona-1, 3, 6, 7, 8, 9; Mammoth Lake-22; Sequoia Park-3, 13, 15; Kern-1.

Southern California: Santa Cruz Island (table 1); Arroyo Seco (Pasadena)-1, 2, 23; Azuza, San Gabriel Canyon (table 1); Dollar Lake-2; Barton Flats-1, 7, 11; Banner (table 1); Julian (table 1); Lake Henshaw-2, 3, 4, 5.

Lower California: Guadalupe (table 1); Santo Tomas (table 1).

Nevada: Charleston-1, 5, 6, 7, 9, 10; Las Vegas-3.

Utah: Cedar City-A.

Idaho: Idaho Falls-2, 3, A (possibly introduced by man).

Nebraska: Scottsbluff-3.

Arizona: Santa Rita Mts.-6.

New Mexico: Carizozo-5, 7.

Texas: Georgetown-1.

*Arrowhead.* British Columbia: Yale-7; Pavilion-5; Kamloops-1; Merritt-2; Okanagan-2, 6, 8; Shuswap-3; Arrowhead-1, 5; Kaslo-4.

Washington: La Grande-2; Olympic-2; Chehalis-4; Chelan-7; Metaline Falls-2, 3.

Idaho: Lake Coeur d'Alene-2, 7, 8; Boise-1, 2; Idaho Falls-1, 2.

Montana: Bitterroot-3, 4, 6, 7.

Wyoming: Big Horn Mts.-5, 6.

South Dakota: Black Hills-3, 5.

Nebraska: Scottsbluff-4, 3.

Oregon: Sisters-6; Newbery Crater-3; Crater Lake-3.

Northern California and Sierra Nevada: Dunsmuir-10; Lassen-16;  
Wawona-1, 7, 8, 9; Mammoth-2, 4, 8, 22; Lake Tahoe-2; Sequoia-11, 15;  
Greenhorn Mts.-11.

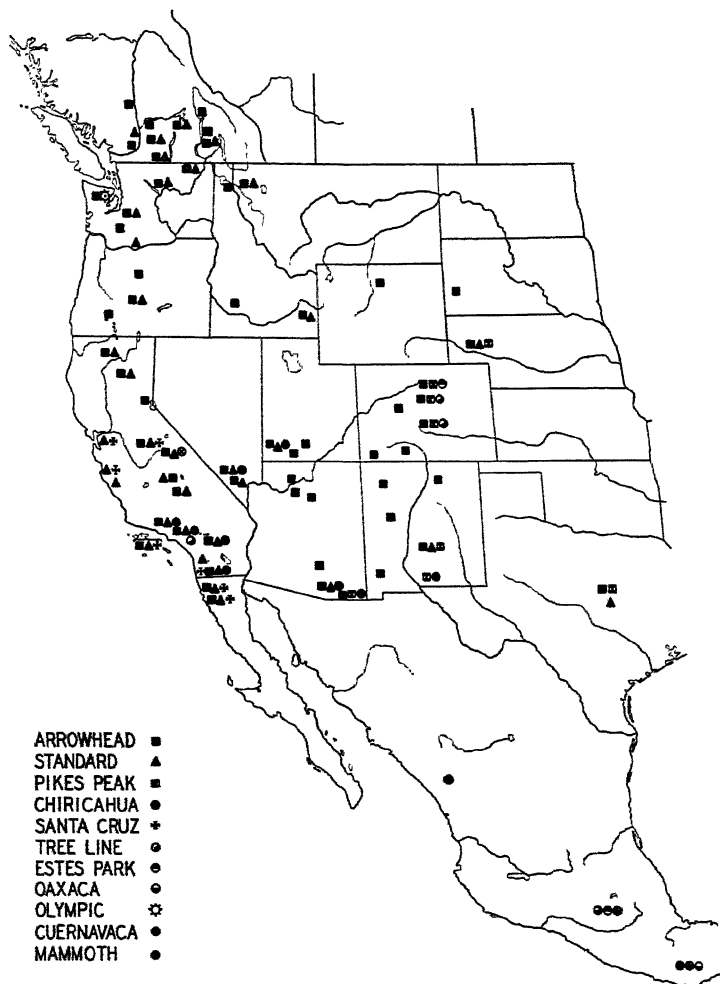


FIGURE 4.—The geographical distribution of the gene arrangements recorded in race A.

Southern California: Santa Cruz (table 1); Arroyo Seco-22, 23; San Gabriel-(table 1); Big Bear-1, 2; Dollar Lake-2, 3; Barton Flats-2, 4, 5, 6, 8, 10, 13, 14, 15; Hidden Springs-1; Banner-(table 1); Julian (table 1); Providence Mts. (table 1).

Lower California: Guadalupe; Santo Tomas (table 1).

Nevada: Charleston-2, 4, 5, 6, 8; Las Vegas-1, 2, 3.

Utah: Cedar City-1, 3, 4, 5, 6, 7, 9, 10, 11, B, C, D; Zion-4, 5; Bryce-4, 5, 6, 7, 8, 9, 10.

Colorado: Aspen-1; Estes Park-2, 3; University Camp-1, 4, 5, 6; Pikes Peak-1, 2, 3, 4, 5, 6, 7; Tree Line on Pikes Peak-2, 3; San Juan Mts.-1, 2, 3, 4, 5, 6, 7, 8, 9; Mesa Verde-1, 3, 4, 5, 6, 7, 8.

Arizona: Grand Canyon North Rim-1, 2, 3; South Rim-1; Flagstaff-1, 2, 5, 7, 8; Gila near Yuma-1, 2; Santa Catalina Mts.-2, 3, 4, 5, 6, 9, 12; Santa Rita Mts.-1, 2, 3, 4, 5, 7; Chiricahua Mts.-2, 4, 6, 7, 8, 10, 11.

New Mexico: Zuni Mts.-2, 3, 4, 5, 6, 7, 8, 9; Taos-1, 3, 4, 6, 7, 8, 9, 10, 11; Magdalena-1, 2, 4, 7, 8, 9; Pinos Altos-1, 2, 3, 4, 7, 8, 9; Carizozo-2, 4, 5, 6, 7, 8, 9, 10.

Texas: Florence-1.

*Chiricahua II.* Arizona: Chiricahua-6.

*Pikes Peak.* Nebraska: Scottsbluff-3, 5, 9.

Colorado: Estes Park-2, 3, 5; University Camp-3, 4, 6; Pikes Peak-1, 2, 4, 5, 6, 7.

New Mexico: Carizozo-9; Carlsbad-1, 2.

Texas: Florence-1, 2.

Arizona: Chiricahua-6.

*Santa Cruz.* California: Wawona-4, 6; Oakland-1; Pacific Grove-1; Santa Cruz (table 1); Banner (table 1); Julian (table 1).

Lower California: Guadalupe; Santo Tomas (table 1).

*Chiricahua I.* California: Arroyo Seco-21; San Gabriel (table 1); Big Bear-1, 2; Dollar Lake-1; Barton Flats-1, 2, 9, 10; Banner (table 1); Julian (table 1); Providence Mts. (table 1).

Lower California: Santo Tomas (table 1).

Nevada: Charleston-2, 4.

Utah: Cedar City-4, E.

Arizona: Santa Rita-1; Chiricahua-3, 5, 4, 9, 11, 12.

New Mexico: Carlsbad-1.

Mexico, Durango: Otinapa-3; Oaxaca: Cerro San Jose-4, 5.

*Cuernavaca.* Mexico, Morelos: Cuernavaca-2, 5, 6, 8; Oaxaca: Cerro San Jose-4, 5.

*Mammoth.* California: Mammoth Lake-2, 8, 19.

*Tree Line.* California: San Gabriel (table 1); Dollar Lake-2.

Colorado: University Camp-1; Tree Line on Pikes Peak-1.

Mexico, Morelos: Cuernavaca-2, 5.

*Estes Park.* Colorado: Estes Park-1, 2.

Mexico, Morelos: Cuernavaca-2, 6.

*Oaxaca.* Mexico, Oaxaca: Cerro San Jose-4.

*Olympic.* Washington: Olympic-2.

## RACE B

*Klamath*. British Columbia: Campbell River-3, 4; Quesnel-5; 150-mile House-5; Pavilion-6; Yale-3; Merritt-4.

Washington: Cape Flattery (La Push)-7; Olympic-5; Quinault-15, 23; Quilcene-4; Seattle-4, 6; The Dalles-7.

Oregon: Reedsport-2; Sisters-9; Crater Lake-2.

California: Klamath-5; Shelter Cove-5; Lassen-2, 8; Mammoth-3, 12; Sequoia-14.

*Standard*. California: Shelter Cove-5; Santa Lucia-11; Nojogui (Santa Barbara)-8; Dunsmuir-8; Lassen-2, 8; Mammoth-1, 3, 4, 7, 9, 10, 11, 12, 13, 15, 17, 20; Sequoia-5, 8, 14, 16.

*Sequoia I*. California: Sequoia-5, 8, 17.

*Wawona*. California: Wawona-4.

*Sequoia II*. California: Sequoia-16.

*Cowichan*. British Columbia: Cowichan Lake-6.

The Standard arrangement is commonest on the Pacific Coast, but occurs sporadically to the limits of the range of the species in the United States. It is nowhere found alone. The Santa Cruz sequence is known from a crescent-shaped area extending from the central Sierra Nevada to the Monterey peninsula, extreme southern California, and Lower California. *D. miranda* occurs in the Puget Sound region (Washington and British Columbia). These three gene sequences seem the best indices of the geographical origin of the group, and they agree in pointing to the Pacific Coast region. With this as a guiding hypothesis we may take up the various families of arrangements in order.

Race B is still confined to the Pacific Coast. Within this area, the standard sequence has been found only in California, and seems to become progressively more frequent to the south. Klamath is the common sequence in race B, occurring practically throughout the range. The other three sequences in race B are known from a single locality each; it should be observed that Sequoia I, the only one of them derived directly from the standard, occurs within the region where standard is frequent. One may surmise that race B arose somewhere in the southern part of its present range, and later spread northward; a conclusion that is not inconsistent with the fact that it is now found further north than race A, and at greater elevations when the two occur in the same region. These relations do, however, suggest caution in accepting the suggested conclusions.

The race A sequences fall into two groups—the Standard and Santa Cruz with their respective derivatives. The Arrowhead arrangement is the commonest one throughout the range of race A in the United States and British Columbia, but has not been found in Mexico (except in northern Lower California). That it is nevertheless of relatively recent origin is sug-

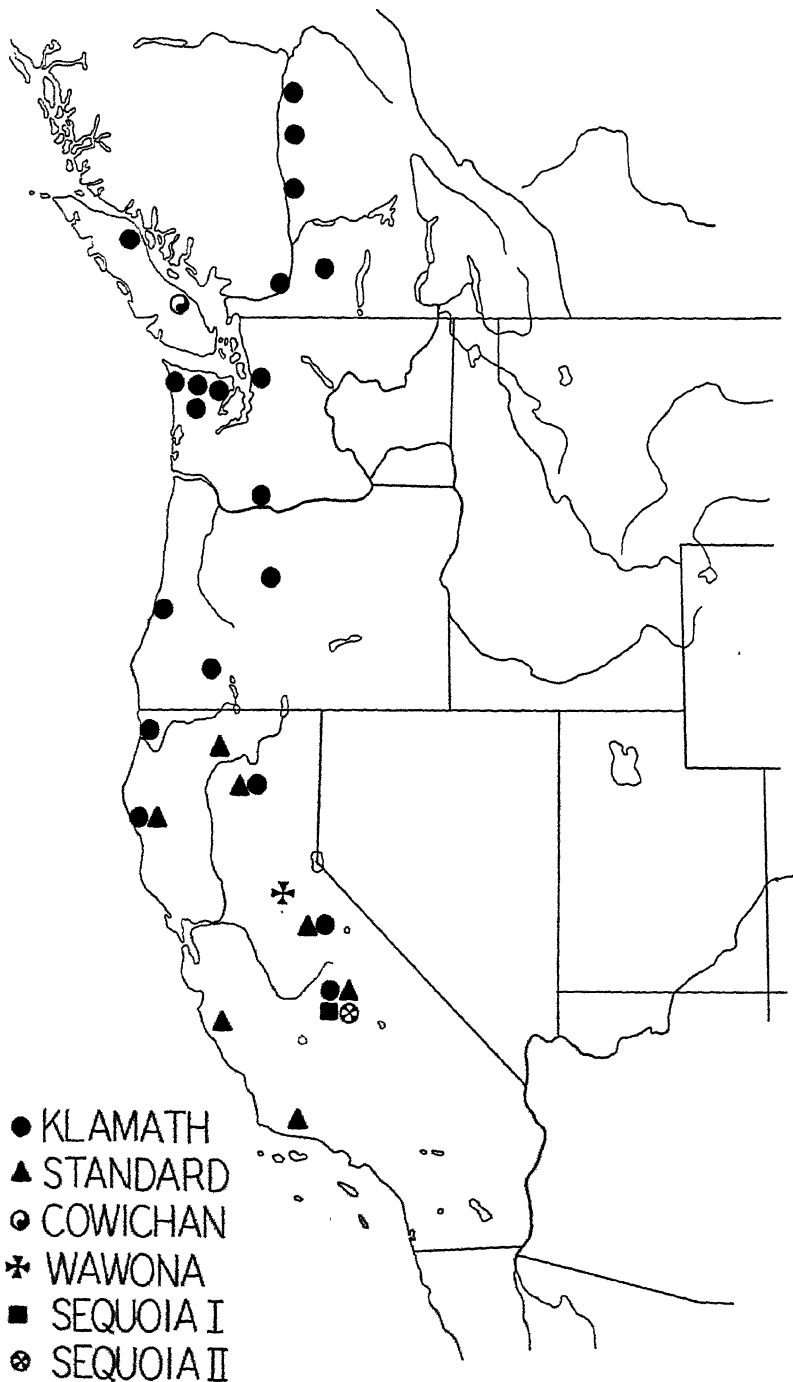


FIGURE 5.—The geographical distribution of the gene arrangements recorded in race B.

gested by the fact that it has given rise to only one new sequence, and that one has been found only in a single stock. Arrowhead is the only sequence that appears to occur over any considerable area unmixed with any others. This area—northern Arizona, western and northern New Mexico, southern Utah, and a portion of southern Colorado—is completely surrounded by regions in which other sequences are frequent. Its historical significance is not clear. The Pikes Peak sequence occurs in the eastern Rocky Mountains with outliers in central Texas and southern Arizona. It presumably arose in this area, where its parent, the Standard, still occurs sporadically.

Sequences derived from Santa Cruz are in general southern in distribution. Tree Line is unusual in two respects, both of which suggest that it is a very ancient sequence. It has given rise to three new sequences (Estes Park, Oaxaca and Olympic), and it has a strikingly discontinuous distribution. It is known from southern California (where it almost overlaps the distribution of Santa Cruz), from the Rocky Mountains of Colorado, and from southern Mexico. Of the three sequences derived from it, Estes Park likewise has a sharply discontinuous range—Colorado and southern Mexico. Olympic is known only from the Olympic peninsula, Washington and Oaxaca only from southern Mexico. The significance of these facts is uncertain; more data from the regions of northern Mexico, Texas, Montana and Idaho are needed. Tentatively, in view of the general southern trend of Santa Cruz derivatives, one may surmise that the occurrence of Tree Line and related arrangements in Colorado and Washington is the result of northward migrations.

Of the other three Santa Cruz derivatives, Mammoth is known only from the eastern slope of the Sierra Nevada, just across the divide from the area where Santa Cruz itself has been recorded. Chiricahua is a widely distributed form, rather frequent in southern California (where it overlaps the distribution of Santa Cruz), and extending from Nevada and southern Utah to southern Mexico. Cuernavaca is thus far known only from southern Mexico. Its occurrence there, together with the probable southern origin of Tree Line, suggests that Santa Cruz is likely to be found in the still unexplored regions of northern and western Mexico.

The inference that the Pacific Coast region is the original home of the species *Drosophila pseudoobscura* finds support in a study of the distribution of other species of this genus. The closest known relatives, aside from *D. miranda*, are evidently *D. obscura*, *tristis*, and *subobscura*—all of which are European. No species from eastern North America or from any other part of the world has yet been found that is structurally so much like *pseudoobscura* as are these. Until collecting is done in northern Asia (a region still almost completely unknown as far as its Drosophilid fauna is concerned), and until more genetic and cytological data concerning the



Palearctic forms are available, it does not seem profitable to speculate concerning the time or the direction of the migrations between Europe and North America. The next most closely related forms seem to be the members of the *D. affinis* group, confined to North America. We have discussed these elsewhere (STURTEVANT and DOBZHANSKY 1937), and came to the conclusion that *D. athabasca* (ranging from Alaska south to Oregon and Colorado) and *D. azteca* (Mexico) formerly occupied a continuous range, and were separated by an eastward extension of the range of the ecologically similar *D. pseudoobscura*—a conclusion which again stands in agreement with the supposition that the latter originated on the Pacific Coast.

To summarize, the presumed history of the group may be sketched as follows, with the proviso that what we have here is merely a working hypothesis, every point being in need of further study. The original American member of the group lived somewhere on the Pacific Coast of the United States, and had the standard or the hypothetical gene arrangement in its third chromosome. Race A of *D. pseudoobscura*, among the known forms, is most like this ancestral type. From it *D. miranda* arose, probably in the northern part of the area. The hypothetical sequence gave rise also to Santa Cruz, this event happening further to the south. Standard gave rise to race B, probably somewhere in California. Race B has remained on the Pacific Coast, gradually spreading northward. The Standard sequence in race A has spread much further east than the other old sequences, but has remained more frequent in its original home near the Pacific. In the Rocky Mountains it has given rise to the Pikes Peak arrangement. One may surmise that the wide range of the Arrowhead sequence is due to its being by chance predominant in the population that played the most important part in the eastward migration; it arose probably in or near the original coastal area.

The Santa Cruz sequence arose very early, probably in California; its greatest development has been in the south. Its derivatives are the only types so far known to occur in Mexico; perhaps the great differentiation occurred there, and was followed by a northward movement (which would then be postglacial in time) of Tree Line and Chiricahua, bringing them into the Rocky Mountains, among the standard derivatives that moved in from the west. One member of this group, the Olympic sequence, has penetrated as far as the Puget Sound region.<sup>1</sup>

<sup>1</sup> In the Puget Sound region are to be found three race A sequences, of which Olympic is only remotely related to the other two; two race B sequences; three different gene sequences in the second chromosome of race B and one in race A; an endemic type of Y chromosome in race B; *Drosophila miranda*; *D. athabasca*. No other area of similar size is known in which diversity approaching this occurs.

THE RELATIVE FREQUENCIES OF DIFFERENT GENE  
ARRANGEMENTS WITHIN A REGION

Most of the data available are based on samples that are too small to give adequate determinations of the frequencies of different sequences in a given region; they must be considered as qualitative rather than quantitative. Quantitative data promise to be interesting, and are now being collected. So far the most satisfactory results are all from southern California and adjacent Lower California; they are shown in table 1.

TABLE 1

*Frequencies of different gene arrangements in wild populations from some localities. The figures indicate the percentage frequencies among the total third chromosomes (n) tested from a given locality.*

| LOCALITY                     | STAND-<br>ARD | ARROW-<br>HEAD | CHIRICA-<br>HUA | SANTA<br>CRUZ | TREE<br>LINE | N         |
|------------------------------|---------------|----------------|-----------------|---------------|--------------|-----------|
| 1. Santa Cruz Island         | 54.7          | 16.7           | —               | 28.6          | —            | 42        |
| 2. Santo Tomas and Guadalupe | 59.4          | 28.1           | 3.1             | 9.4           | —            | 32        |
| 3. East of Julian            | 45.2          | 25.8           | 25.8            | 3.2           | —            | 31        |
| 4. West of Julian            | 47.0          | 35.3           | 11.8            | 5.9           | —            | 17        |
| 5. Banner                    | 57.1          | 26.2           | 14.3            | 2.4           | —            | 42        |
| <b>Total 3, 4, and 5</b>     | <b>51.1</b>   | <b>27.8</b>    | <b>17.8</b>     | <b>3.3</b>    | <b>—</b>     | <b>90</b> |
| 6. San Gabriel Canyon 1936   | 14.8          | 27.8           | 40.7            | —             | 16.7         | 54        |
| ditto, 1937                  | 57.5          | 27.7           | 10.6            | —             | 4.3          | 47        |
| 7. Providence Mts.           | 8.0           | 82.0           | 10.0            | —             | —            | 300       |

The two collections from Julian were made on two successive days, in October, 1936, at points about four miles apart; that from Banner was made in the following April at a point about seven miles east of the more eastern Julian locality, and at several thousand feet less elevation. These collections appear to have had substantially the same frequencies of the four gene sequences concerned; they are little if any different from the Lower California populations (Santo Tomas, Guadalupe), which were collected in April 1936 at points somewhat over fifty miles from Julian but connected by an area that is presumably inhabited throughout by large numbers of *pseudoobscura*. The other four populations shown in table 1 all appear to be significantly different from these and from each other. In the case of the two from San Gabriel Canyon this is of particular interest, since these represent collections from a single small locality, made in November 1936 and in April 1937. Evidently the proportions of the gene sequences concerned changed markedly during the winter, though the same four occur in each collection. It should be noted that this locality is much disturbed by man, being close to permanent dwellings and also a popular week-end resort. It is possible that such great fluctuations are not

usual under natural conditions; only further studies can decide this point. In general, the material on which the present investigation is based has been collected in localities as far as possible removed from human habitation.

#### DISCUSSION

As shown above, the overlapping inversions may be used as a tool for the study of historical problems. In this connection it is necessary to consider the question whether the same inversion may arise repeatedly. It is evidently of importance to decide how safe it is to assume that two chromosomes having a given gene arrangement are descended from a common ancestor having the same arrangement.

One of us (STURTEVANT 1931) had concluded that such recurrences are frequent; we are now of the contrary opinion. The earlier argument was based on the wide geographical range of specific gene sequences in *D. melanogaster*; we should now interpret the facts as indicating a high degree of constancy in gene sequence. GERSHENSON (1930) and GRÜNEBERG (1936) have reported cases of "reversion," where a sequence has given rise to the ancestral sequence from which it was derived. We are not completely convinced that other possible explanations (such as crossing over or contamination) have been excluded in these cases. In general, the chance of the occurrence of a new inversion, both of whose ends shall coincide with those of a previous one, seems very remote. A few instances are known in which one of the ends in two inversions seems so to coincide, but we are not acquainted with any examples in which such a coincidence has been fully demonstrated by a detailed comparison in salivary gland chromosomes.

From our data it appears that the end-points of the inversions in the third chromosome of *D. pseudoobscura* are not distributed at random through the chromosome. Thus, several breakages are known in the short sections 76 and 77, and only two in the interval including sections from 63 to 67 inclusive which constitutes more than one third of the total length of the chromosome. But even in the sections in which the breakages are numerous there are no unequivocal cases of exact correspondence—clearly the usual thing is that the inversion-points, even when they lie in the same region, are not identical.

We can only conclude that, while recurrences of the same inversion must be recognized as possible, they are too improbable to be postulated in any given case.<sup>2</sup> We have used a number of strains that were homozygous for

<sup>2</sup> One reservation may be made here, however. In many cases we have identified sequences only by the configurations seen in heterozygotes. There exists a remote possibility that in some instances we may be dealing with "mimic" sequences, differing by a few discs from those with which they are identified. In every instance where a sequence has a markedly discontinuous dis-

sequence, and have kept them for several years. It is clear that these, like similar strains of other species, have not undergone changes in sequence. The uniformity with which the Arrowhead sequence is found in the southern Rocky Mountain region may be taken as a further indication of the rarity with which new inversions occur.

If new inversions arise rarely, it becomes of interest to inquire how some of them come to be established in wild populations inhabiting large territories. We have no answer to give to this question, other than to say that inversions are in this respect not different from gene mutations and do not require any different theory—except in one respect. It has been questioned whether gene mutations that are wholly neutral in relation to natural selection can be supposed to exist. Inversions would seem to come closer to such a neutral condition than any other genetic character, if one neglects the possibility of position effects that they may produce. In general, heterozygosis for inversions decreases the amount of crossing over, and this may be of selective value in connection with heterosis effects. The magnitude of this effect of inversions cannot be evaluated satisfactorily at present, though experiments under way in this laboratory should enable us to reach definite conclusions.

On the other hand, heterozygosis for some inversions may have a slight unfavorable effect. Long inversions, such as Pikes Peak/Standard, should give an appreciable frequency of crossing over, and there should be some mortality among the offspring as a result (STURTEVANT and BEADLE 1936). When two sequences differ by two inversions whose ends are not too different, crossovers should occur giving inviable or poorly viable zygotes (STURTEVANT and BEADLE, loc. cit.). Perhaps the most likely combination for this effect is Pikes Peak/Arrowhead. It would seem that the Pikes Peak sequence should be at a selective disadvantage whenever it occurs in the same population either with Standard or with Arrowhead. And yet just these sequences are the ones with which Pikes Peak is most often associated; it is probable that it does not exist in any considerable area that is free of the Arrowhead sequence. One is led to wonder how effective are slight increases or decreases in mortality in determining the fate of chromosome structures or genes in wild populations of this species. The observation that autosomal lethals are present in approximately one-sixth of the third chromosomes of wild strains gives further point to this question.

*Drosophila pseudoobscura* is, as species go, relatively constant in external morphology. We have had under observation some hundreds of wild

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tribution, representatives from the different regions have been tested directly, by studying the salivary gland chromosomes of homozygotes. A fairly large number of such direct tests has been made also for sequences showing continuous distribution. No "mimics" have been found. The reservation made here is not intended to cast doubt on the validity of the distribution maps shown in figures 4 and 5.

strains, from localities scattered from British Columbia to southern Mexico and from the Pacific to Texas. None of these have been distinct enough so that we should feel certain of distinguishing unlabeled cultures from the external appearance of the flies. Yet this same material has been found to show striking heterogeneities in a variety of characters. The "strength" (testis size in  $B \text{ } \varnothing \times A \text{ } \sigma$  hybrids) has been shown to be extremely variable (DOBZHANSKY and BOCHE 1933; also unpublished data); seven different types of Y chromosomes occur (DOBZHANSKY 1935b, 1937); the present account shows the existence of a wide diversity in gene arrangements. In addition, unpublished results show that there are frequent and marked differences in the modifiers affecting bristle number, in the genes affecting the number of male offspring produced by A-B hybrid females, and in the ease with which the various strains cross with *Drosophila miranda*.

A comparison of the variability of the gene arrangement in the third chromosome with that of the structure of the Y chromosome leads to results that are suggestive. Each type of Y chromosome has a geographical distribution that roughly corresponds to that of one or more of the third chromosome sequences, as follows:

| Y chromosome | Third chromosome               |          |
|--------------|--------------------------------|----------|
| Type I       | Oaxaca, Cuernavaca, Chiricahua | } Race A |
| Type IV      | Standard                       |          |
| Type V       | Arrowhead                      |          |
| Type VI      | Pikes Peak, Chiricahua         |          |
| Type VII     | Estes Park, Tree Line          |          |
| Type I       | Klamath                        | } Race B |
| Type II      | Cowichan                       |          |
| Type III     | Standard, Sequoia              |          |

These agreements are rather rough, but they may be made into a consistent scheme if it be assumed that the original Y chromosome was Type I (which is, morphologically, the largest of all, and looks as though all the others could be derived from it by losses of parts). This Y was, on such a basis, originally associated with the Standard, "hypothetical," or Santa Cruz sequences, probably with all three. In race A it has persisted only in part of the region that is occupied by Santa Cruz derivatives; in race B it has persisted throughout the range, but has become less common to the south.

Type IV of Y chromosome may be supposed to have arisen from Type I by loss of most of the short arm, Type V from Type IV by loss of a portion of the long arm. Types II, III, and VII perhaps came directly from Type I; Type VI, because of its structure, seems more likely to have come from I than from IV or V, and may therefore be tentatively associated with Chiricahua rather than with Pikes Peak.

All these suggestions are to be considered as speculative; they are presented here as an example of the way in which such historical problems may be approached with this material.

#### SUMMARY

1. Strains of *Drosophila pseudoobscura* coming from the same or from different geographical localities are frequently dissimilar with respect to the gene arrangement in their chromosomes. The variations in the gene arrangement are due to inversion of chromosome sections, no translocations or other chromosome aberrations having been found.

2. The third chromosome is the most variable one, seventeen different gene arrangements having been recorded; the second chromosome with six follows next; next are the right limb of the X chromosome with three, and the left limb of the X and the fourth chromosomes with two arrangements each. The fifth chromosome has not been sufficiently studied.

3. Each gene arrangement occurs in a definite geographical area. Some arrangements have been recorded so far only in a single locality, while others are distributed fairly widely (figs. 4 and 5). In many localities the populations are mixed, four or more gene arrangements being present. However, none of the seventeen arrangements known in the third chromosome is present in the entire distribution region of the species.

4. A chromosome once changed by an inversion may undergo another change due to a second inversion. The second inversion may be independent, included, or overlapping the first (fig. 1). Overlapping inversions are especially interesting, since they allow conclusions to be drawn regarding the historical sequence of changes in the chromosome involved. With three gene arrangements, A, B, and C related as overlapping inversions, the phylogenetic series is  $A \longleftrightarrow B \longleftrightarrow C$ , but not  $A \longleftrightarrow C$  (cf. pages 31-34).

5. The gene arrangements recorded in the chromosomes of *D. pseudoobscura* are as a rule related as overlapping inversions. This permits the drawing of phylogenetic charts representing the historical sequence of changes in the chromosomes in question (fig. 3 for the third, p. 48 for the second chromosomes).

6. The overlapping inversion method itself does not permit the determination of the original (ancestral) gene arrangement in a given chromosome. Among the gene arrangements recorded in the third chromosome there exist however three arrangements (Standard, "hypothetical," and Santa Cruz, see fig. 3) which on other grounds are likely to be ancestral ones.

7. The overlapping inversion method is applicable for comparison of distinct species as well as strains of the same species. It is shown that

*Drosophila miranda* is more closely related to race A than to race B of *D. pseudoobscura*.

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# CYTOGENETIC STUDIES IN PAEONIA I. THE COMPATIBILITY OF THE SPECIES AND THE APPEARANCE OF THE HYBRIDS

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Received September 4, 1937

## INTRODUCTION

SINCE 1916, the senior author has carried on an extensive series of crossing experiments in the genus *Paeonia*, with the object both of obtaining new horticultural forms and of securing evidence concerning the interrelationships of the species and the processes of evolution within the genus. Some accounts of the hybrids have already been published (SAUNDERS 1928, 1933a, 1933b). The junior author began cytological work on the species and hybrids in 1932, continuing that started by the late Dr. G. C. HICKS, whose work along with additional data obtained by the junior author has also been published (HICKS and STEBBINS 1934). Since that time the cytological as well as the morphological study of the hybrids has progressed steadily, except that it was somewhat interrupted by the departure of the junior author for California in 1935.

The following limitations prevent a cytogenetic study of this genus comparable to that carried on in other genera, such as *Crepis*, *Nicotiana*, and *Datura*. Peony seeds are slow of germination, and the plant takes several years to mature; from the date of hybridization to the season of first blooms is usually a gap of six or seven years. This makes it difficult for one individual to raise many successive generations. An added difficulty is that most interspecific hybrids in *Paeonia* are completely sterile for at least two or three years after they first begin to bloom; older plants of almost all the hybrids, when they have established themselves as strong clumps, set occasional seeds, usually not more than one or two to an entire plant. The size of the plants and the cost of their care through so long a period of years also make large scale experimentation for statistical data impracticable. There are in this collection at present several thousand hybrid plants of blooming age, representing more than thirty distinct strains. The range of attempted crossings was much wider, but as was to be expected many of them persistently failed to produce seed.

## SOURCE OF THE MATERIAL

In any genus that contains a number of cultivated species, interspecific relationships are likely to be obscured by selection and hybridization under cultivation. For this reason, particular attention has been given to the source of the material used, and every effort has been made to determine



the genetic similarity of the various clones used to wild biotypes. Fortunately for this purpose, the clones of *Paeonia* are very long lived, and the species are relatively infrequently propagated by seed, so that forms which have been in cultivation for many years are only a few generations removed from their wild prototypes. Furthermore, with a few notable exceptions, interspecific hybrids in the genus are so sterile that they can readily be identified, and in most cases would be very unlikely to contaminate the pure species by backcrossing. Hence the cultivated clones that differ from the wild stocks of the same species are in most cases either hybrids between different varieties or biotypes, or strains resulting from the repeated selection of favorable mutations. The most notable exceptions to this rule, the tetraploid European species and garden forms, have for this reason been given relatively little attention in the general study of interspecific relationships in the genus, although significant evidence has been obtained as to the probable mode of origin of the group as a whole. This will be presented more fully in a later paper.

The following table describes the origin and character of the clones most frequently used for hybridization. The letter *r* after the designation of the source indicates that roots were obtained, the letter *s* that seed was received, from which seedlings were grown by the senior author. The approximate date of introduction into cultivation of each form is given, based on such published records as are available, while the number of generations which the particular clone used in hybridization is removed from the wild plant is estimated from notes received from the various horticultural firms, and from the frequency with which the form is grown and propagated. In the final column the abbreviation "herb." indicates that the clone used has been matched with authentic herbarium specimens of wild plants, while "ill." indicates that it compares closely with a published illustration accompanying the original or another authentic description of the form. The taxonomic status of the forms designated with a star (\*) is discussed elsewhere (STEBBINS 1938). The name Van Tubergen refers to C. G. VanTubergen Ltd., Haarlem, Holland; Vilmorin, to Vilmorin et Cie., Paris; Glasnevin to Glasnevin Botanical Gardens, Dublin Ireland; and Barr to Barr and Sons, Ltd., London.

#### COMPATIBILITIES OF THE SPECIES

Although the number of matings made of any particular cross was in most cases not large enough so that accurate quantitative data could be secured concerning the degree of success, it was possible to group them into four classes:

- I. Hybrids obtained as in crosses between individuals of the same variety (about 80-100 percent seed setting).

| NAME   | SOURCE                                   | NATIVE HABITAT       | DATE INTRODUCED | GENERATIONS FROM WILD PLANT | COMPARED   |
|--|--|----------------------|-----------------|-----------------------------|------------|
| <i>P. Delavayi</i> Franch. var. <i>luteo</i><br>(Franch) Finet & Gagnep                | Van Tubergen—r                           | S W. China           | 1885            | 2-3                         | herb.      |
| <i>P. suffruticosa</i> Andr  | various                                  | N.W. China           | ?               | ∞                           | o          |
| <i>P. albiflora</i> Pall.<br>The Bride (single)  | various                                  | N. China             | ?               | ∞                           |            |
| Clairette (single)   | various                                  | Manchuria            | ?               | ∞                           | o          |
| Whitleyi major (single)  | various                                  | Siberia              | ?               | ∞                           | o          |
| Primivere (double)   | various                                  |                      | ?               | ∞                           | o          |
| James Kelway (double)  | various                                  |                      | ?               | ∞                           | o          |
| <i>P. anomala</i> L.<br>typical  | Highland Park<br>Rochester, N.Y.—r       | Siberia              | 1750            | ?                           | herb. ill. |
| <i>Veitchii</i> *  | Vilmorin—r                               | W. China             | 1909            | 1-2                         | herb.      |
| <i>Woodwardii</i> *  | Vilmorin—r                               | W China              | 1920            | 1-2                         | herb.      |
| <i>Beresowskii</i> *   | Glasnevin—r<br>Leningrad B G.—s          | W China              | 1920            | 1-2                         | herb.      |
| vars.  | H.A. Hesse<br>Weener, Germany            | W. China             | ?               | ?                           | herb.      |
| <i>P. Emodi</i> -Wall.<br>typical  | Glasnevin—r<br>Hyde Park<br>London—s     | Himalaya             | 1840-<br>1860   | 2-6                         | herb. ill. |
| <i>P. tenuifolia</i> L.<br><i>P. trilateralis</i> Pall                                 | private garden—r                         | S E. Europe          | 1750            | ?                           | herb. ill. |
| typical  | Barr—r                                   | S E. Europe          | 1810            | ?                           | herb. ill. |
| <i>Mlokoszewitschii</i> (Lomak.)*  | Van Tubergen—r<br>and own—s              | Caucasus             | 1895            | 2-3                         | ill.       |
| <i>P. Broteri</i> Boiss*   | Barr—r                                   | Spain,<br>Portugal   | 1880-<br>1885   | 2-5                         | herb. ill. |
| <i>P. obovata</i> Maxim  | Sakata & Co<br>Yokohama—s                | Japan                | 1890-<br>1900   | 1-2                         | herb       |
| <i>P. tomentosa</i> Stapf. (= <i>P. macrophylla</i> of auth not Lomak, cf. Stapf 1931) | Van Tubergen—r                           | Caucasus             | 1900-<br>1908   | 3-5                         | ill.       |
| <i>P. coriacea</i> Boiss   | Cent. Expt.<br>Farm, Ottawa,—r<br>Canada | S. Spain,<br>Morocco | 1870-1890       | ?                           | o          |
| <i>P. Wiltmanniana</i> Hartw.<br>typical   | Van Tubergen                             | N. Persia            | 1840-1845       | 3-4                         | o          |
| <i>Wilmottiae</i> (Stapf)*   | J. C. Allgrave<br>Langley, England—r     | W. China             | 1908            | 1                           | o          |
| <i>P. corallina</i> Retz.*   | Vilmorin—r                               | S. Europe            | ?               | ∞                           | herb. ill. |
| <i>P. officinalis</i> L.<br>single crimson   | own—s                                    | Europe               | ?               | ∞                           | o          |
| rubra plena  | various—r                                | Europe               | ?               | ∞                           | o          |
| Otto Froebel   | various—r                                | Europe               | ?               | ∞                           | o          |

II. Hybrids obtained with some difficulty (2-80 percent seed setting).

III. Hybrids obtained with great difficulty (less than 2 percent of normal seed setting).

IV. Hybrids not obtained.

*Compatibilities between the diploid species.* These are illustrated by the chart, figure 1. This shows that the degree of compatibility between two forms is a good criterion for the delimitation of species and subgenera. In every case where the compatibility was perfect, the parents were closely related and the hybrid fertile, indicating that the cross was between different varieties of the same species. On the other hand, crosses between

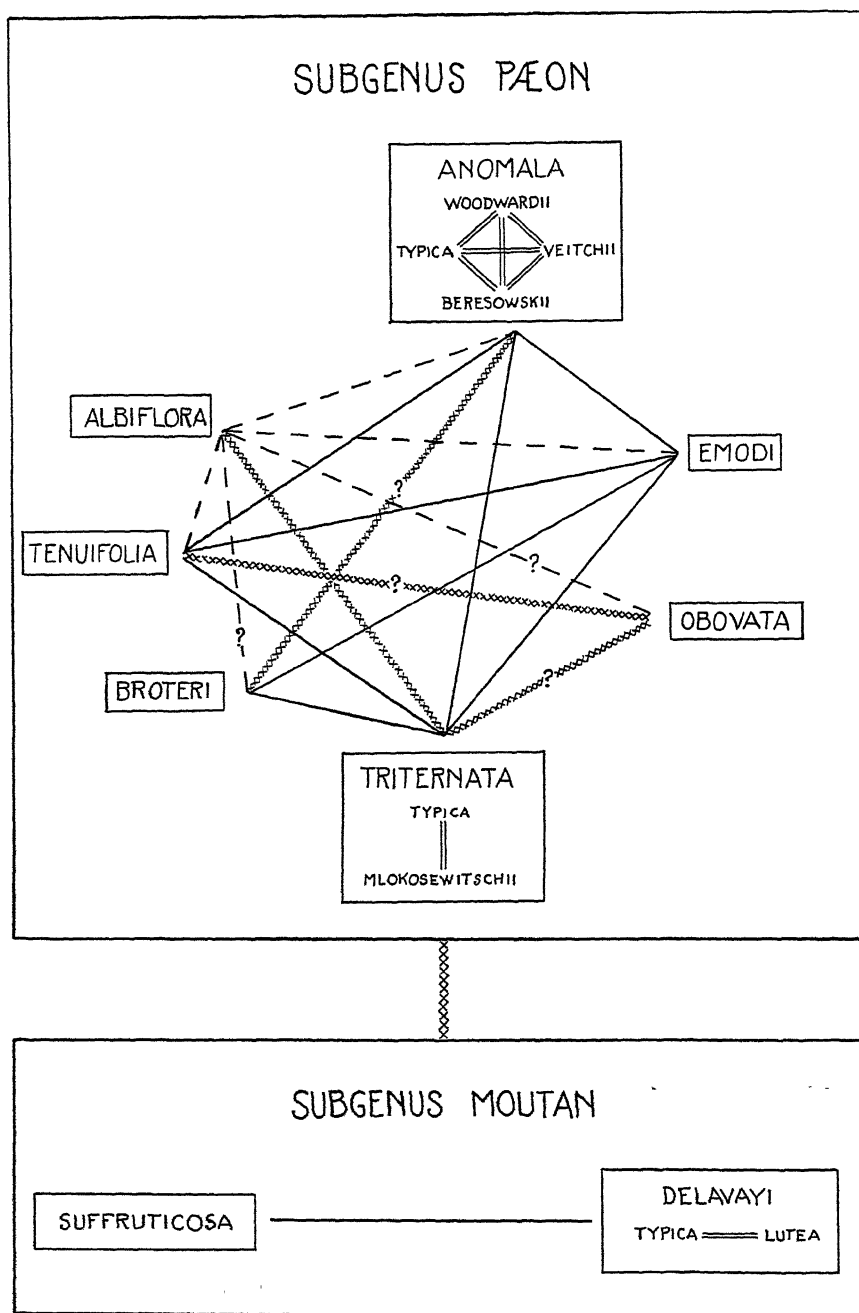


FIGURE 1. (See opposite page for description.)

members of different subgenera have never been successful, while in only one case (*P. albiflora* × *P. triternata Mlokoewitschii*) has a cross between two diploid members of the same subgenus been unsuccessful after a large number of attempts.

On the other hand, compatibility has been of no value whatever in determining the interrelationships within a single subgenus. For instance, *P. anomala* is fairly similar in leaf, sepal, and other floral characteristics to *P. albiflora*, but crossing between the two is very difficult; on the other hand it is relatively easy between *P. anomala* and *P. triternata Mlokoewitschii* or *P. tenuifolia*, both of which are much more different in their appearance from *P. anomala* than is *P. albiflora*.

*Compatibilities between the tetraploid species.* The tetraploid species, in contrast to the diploid, are uniformly perfectly compatible with each other, and most of them intercross naturally when placed side by side in the garden. Although many of the hybrids thus obtained are partially or wholly sterile, this ability for free intercrossing is undoubtedly an important cause of the taxonomic complexity of these species.

*Compatibilities between diploid and tetraploid species.* Only two tetraploid species groups have been tested sufficiently, *P. officinalis* (including the various varieties and horticultural forms mentioned above) and *P. tomentosa*. The compatibility of these two with the various diploid species is given in table 1. A striking fact brought out by this table is that *P. albiflora*, which is very difficult to cross with the other diploid species, crosses relatively easily with the tetraploids. In this series also compatibility was not the greatest between species closely similar morphologically. For instance, *P. triternata Mlokoewitschii* and *P. tomentosa*, two species taxonomically very close to each other, cross with such difficulty that out of 54 pollinations only eight F<sub>1</sub> plants have been obtained and yet *P.*

FIGURE 1.—Chart illustrating the compatibilities of the diploid species of *Paeonia*. With the exception of *P. Emodi* and *P. obovata*, each species within the rectangle representing the subgenus *Paeon* occupies a position between its two closest relatives; hence the closest taxonomic relationship is between adjacent species, and the greatest divergence is between species opposite each other. The lines between the rectangles representing subgenera, species, and subspecies are explained as follows:

===== Hybrids obtained as easily as in crosses between individuals of the same subspecies, fertile.

————— Hybrids obtained with some difficulty (2 percent or more of normal seed setting) and sterile.

- - - - - Hybrids obtained with great difficulty (less than 2 percent of normal seed setting), sterile.

XXXX Hybrids not obtained.

A ? indicates that the number of attempts as yet made is insufficient to determine definitely the nature of the cross. The absence of a connection between two rectangles indicates that the cross between these species has not been attempted.

*tomentosa* crosses relatively easily with the very distantly related species, *P. albiflora*. Furthermore, the compatibility of *P. albiflora* with some races of *P. officinalis*, such as the horticultural var. *lobata*, is very high, while other races of *P. officinalis* can be crossed only with great difficulty. This difference is illustrated in table 1, where *P. albiflora* appears in several places under *P. officinalis*.

The other unexpected fact is the behavior of reciprocal crosses between diploid and tetraploid species of this genus. Practically all such crosses

TABLE 1

Table illustrating the compatibilities of two tetraploid species, *Paeonia officinalis* and *P. tomentosa*, with the diploid species.

|   | <i>officinalis</i>   | <i>tomentosa</i>  |
|---|--|---|
| I. Hybrids easily obtained                  | <i>albiflora</i> *   |   |
| II. Hybrids obtained with some difficulty   | <i>albiflora</i> *   | <i>albiflora</i> ♀  |
| III. Hybrids obtained with great difficulty | <i>albiflora</i> *<br><i>tenuifolia</i><br><i>triternata</i>       | <i>triternata</i><br><i>albiflora</i> ♂                                       |
| IV. Hybrids not obtained                    | <i>Delavayi</i><br><i>suffruticosa</i><br><i>anomala</i>           | <i>anomala</i><br><i>tenuifolia</i><br><i>Delavayi</i><br><i>suffruticosa</i> |
| Not attempted                               | <i>Emodi</i><br><i>obovata</i><br><i>Broteri</i><br><i>Brownii</i> | <i>Emodi</i><br><i>obovata</i><br><i>Broteri</i><br><i>Brownii</i>            |

\* See text for explanation.

have been made with a tetraploid species as the pollen parent, and a diploid, usually *P. albiflora*, as the ovulate parent. The results of reciprocal hybridization show that the cross between *P. albiflora* and *P. tomentosa* can be made much more easily with the former as the ovulate parent (SAUNDERS 1933b). This is contrary to the rule in most other genera (WATKINS 1932, and many other cases). In the other diploid-tetraploid cross listed, *P. albiflora* × *officinalis*, there was no significant reciprocal difference in compatibility. No explanation for this difference in behavior between the *P. tomentosa* and *P. officinalis*, or for the unusual situation in the hybrid between the former and *P. albiflora* can as yet be given. It may be noted, however, that the seeds of *Paeonia* developed a copious endosperm, so that the disharmony between embryo and endosperm development postulated by WATKINS would be expected to hold here.

## APPEARANCE OF THE HYBRIDS

In the appearance of the hybrid populations in general, the variability of the  $F_1$  plants from the same cross is most striking. This is particularly true in crosses which involve as one parent *P. albiflora*, *P. suffruticosa*, or some member of the complex of *P. officinalis*. Since these three species have all been cultivated for centuries and possess a large number of horticultural varieties, the variation in the  $F_1$  populations probably reflects the heterozygous character of one or both parents. Nevertheless, sometimes

TABLE 2

The character of the sepals in diploid and tetraploid species and their hybrids. The character of the innermost sepal is expressed by the presence (+) or the absence (—) of a strong midrib which persists to its apex, and the length of the terminal mucro, if present (drawings, in figures 2 and 3).

| SPECIES<br>OR HYBRID                                      | SOMATIC<br>CHROMOSOME<br>NUMBER | USUAL<br>NUMBER<br>OF SEPALS | INNERMOST SEPAL           |                       |
|---|---------------------------------|------------------------------|---------------------------|-----------------------|
|   |                                 |                              | CHARACTER<br>OF<br>MIDRIB | LENGTH<br>OF<br>MUCRO |
| <i>P. tenuifolia</i>                                      | 10                              | 7                            | —                         | 0                     |
| <i>P. triternata</i> Mlokosewitschii                      | 10                              | 4                            | —                         | 0                     |
| <i>P. anomala</i> Veitchii                                | 10                              | 6                            | +                         | 5-7 mm                |
| <i>P. anomala</i> Woodwardii                              | 10                              | 6                            | +                         | 1.5-2 mm              |
| <i>P. albiflora</i>                                       | 10                              | 7                            | +                         | 0.5-1.5 mm            |
| <i>P. officinalis</i>                                     | 20                              | 5                            | +                         | 0                     |
| <i>P. tomentosa</i>                                       | 20                              | 4                            | —                         | 0                     |
| <i>P. tenuifolia</i> × <i>an. Veitchii</i>                | 10                              | 7                            | +                         | 2 mm                  |
| <i>P. tenuifolia</i> × <i>an. Woodwardii</i>              | 10                              | 7                            | +                         | 1.5-2 mm              |
| <i>P. tenuifolia</i> × <i>albiflora</i>                   | 10                              | 7                            | +                         | 1-1.2 mm              |
| <i>P. tri. Mlokosewitschii</i> × <i>P. an. Veitchii</i>   | 10                              | 6                            | +                         | 1.5 mm                |
| <i>P. tri. Mlokosewitschii</i> × <i>P. an. Woodwardii</i> | 10                              | 6                            | +                         | 0.2 mm                |
| <i>P. albiflora</i> × <i>officinalis</i>                  | 15                              | 6-7                          | 18+<br>16—                | 0-0.7 mm              |
| <i>P. albiflora</i> × <i>tomentosa</i>                    | 15                              | 4-5                          | —                         | 0                     |

the variation within a single population transcends that of either of the parent species. For instance, among the  $F_1$  hybrids of *P. albiflora* × *tomentosa*, occasional dwarf plants with strap-shaped petals appear. These may be sibs of perfectly normal plants, or even of plants which show "hybrid vigor" in the great size of their leaves. A similar situation prevails in the crosses of *P. albiflora* × *P. officinalis*, *P. albiflora* × *P. tenuifolia*, and *P. Delavayi* × *P. suffruticosa*. In crosses between species possessing few horticultural varieties, and presumably more homozygous, the  $F_1$  populations are much more uniform.

Although the  $F_1$  hybrids of each cross are intermediate between their parents in nearly every characteristic, definite trends of dominance and recessiveness could be noted in a few, as follows:

*The sepal characters.* As is explained in another publication (STEBBINS 1938), the difference between the sepals of the species is one of the most significant taxonomic characters in the genus, and shows a progression from a phylogenetically primitive to an advanced condition. Since this character may be most accurately represented by the number of sepals and the character of the innermost sepal, a table showing these in the crosses involving sepal differences is presented in table 2.

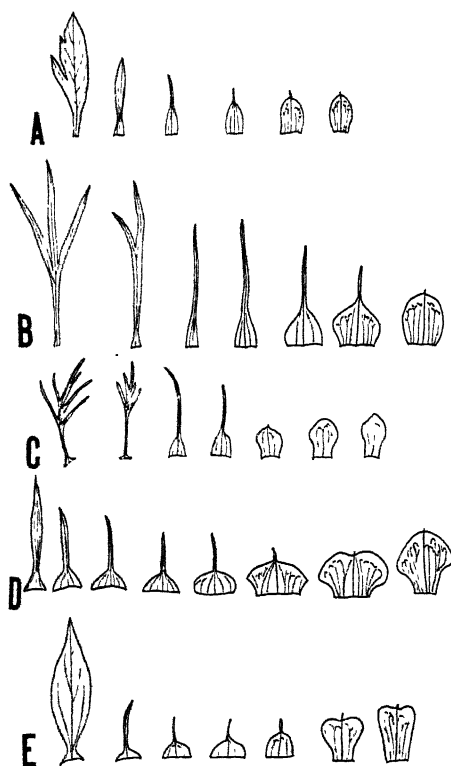


FIGURE 2.—The sepals of: A. *Paeonia anomala Woodwardii*, B. *P. tenuifolia* × *Woodwardii*, C. *P. tenuifolia*, D. *P. albiflora* × *tenuifolia*, E. *P. albiflora*. All  $\times \frac{1}{2}$ .

This table shows that, while the sepals of the hybrids are to a certain extent intermediate between those of their parents, all of the diploid hybrids are more nearly like their more primitive parent, that is, that one which retains the appendage on the innermost sepal. Hence we may conclude that the genes for the more primitive type of sepals are more or less, though not completely, dominant over those for the more advanced type. The various sepal types involved are illustrated in figure 2 and elsewhere (STEBBINS 1938).

Noteworthy exceptions to this rule are found, to be sure, in crosses involving one of the tetraploid species, for example, *P. officinalis* or *P.*

*tomentosa*. In the *P. albiflora* × *tomentosa* series, although about fifty  $F_1$  plants were examined, none was found in which the midrib of the innermost sepal extended to its apex (fig. 3). One may conclude from this fact that the two diploid genomes which make up the tetraploid set of *P. tomentosa* both contain genes for the absence of a midrib and mucro, and that therefore the double number of genes for this character present in the hybrid has overcome the normal dominance of the midrib-mucro genes. The hybrids between *P. albiflora* and *P. Wittmanniana* as well as *P. coriacea*, two other tetraploid species closely related to the diploid types which lack the midrib and mucro, are quite similar, although in these cases only two or three plants were examined.

TABLE 3  
*The inheritance of side buds.*

| PARENTS                    |  | HYBRID  |      |
|----------------------------|--|---------|------|
| <i>Pollen</i>              | <i>Ovulate</i>                             |         |      |
| <i>P. lutea</i>            | + × <i>P. suffruticosa</i>                 | —       | (+)  |
| <i>P. tenuifolia</i>       | — × <i>P. anomala</i> Veitchii             | (+)     | —    |
| <i>P. tenuifolia</i>       | — × <i>P. anomala</i> Woodwardii           | +       | (+)  |
| <i>an. Veitchii</i>        | (+) × <i>P. triternata</i> Mlokosewitschii | —       | —    |
| <i>an. Mlokosewitschii</i> | — × <i>an. Woodwardii</i>                  | +       | +    |
| <i>P. albiflora</i>        | + × <i>P. tenuifolia</i>                   | —       | +    |
| <i>P. albiflora</i>        | + × <i>P. officinalis</i>                  | —       | +    |
| <i>P. albiflora</i>        | + × <i>P. coriacea</i>                     | —       | —    |
| <i>P. albiflora</i>        | + × <i>P. tomentosa</i>                    | — $F_1$ | +, — |
|                            |  | $F_2$   | +, — |

The  $F_1$  plants of *P. albiflora* × *P. officinalis* are quite different. Eighteen of them had sepals more nearly resembling those of *P. officinalis* while sixteen were nearer to *P. albiflora* in this character. These results suggest that the genome of *P. officinalis*, in contrast to those of the tetraploids mentioned above, is heterozygous for the sepal character, and that segregation has occurred. This indicates a difference between the two original diploid genomes of this species. The importance of this fact in connection with the origin of *P. officinalis* and its relatives will be emphasized in a later publication.

*The number of flowers per plant.* The more primitive species of Paeonia—*P. Delavayi*, *P. Emodi*, most varieties of *P. anomala*, and *P. albiflora*—produce, in addition to the large bud that terminates the main axis, one to four small side branches that bear smaller buds and flowers. The other species, more advanced in their floral characteristics, bear a single flower on each stem.

Table 3 shows the inheritance of this character in interspecific crosses. A + indicates the presence of side buds, a —, their absence. A (+) in-



dicates that the side branches are weakly developed, and frequently fail to produce flowers.

This table shows that, as in the case of sepal characteristics, the hybrids resemble more nearly the primitive parent, although here exceptions are observed. One among the diploid species is the hybrid *P. anomala Veitchii*  $\times$  *P. triternata Mlokosewitschii*. Its significance is reduced, however, by the fact that side branches are often weakly developed or absent in

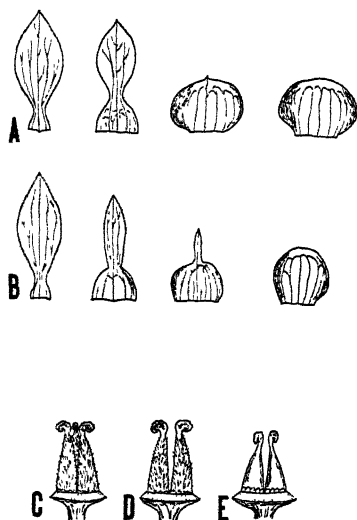


FIGURE 3.—The sepals of: A. *Paeonia tomentosa*; B. *P. albiflora*  $\times$  *tomentosa*, "Seraph." The gynaeceia, at anthesis, of C. *P. tomentosa*; D. *P. albiflora*  $\times$  *tomentosa*, "Seraph"; E. *P. albiflora* (Vilmorin seedling). A, B,  $\times \frac{1}{2}$ , C-E  $\times 1$ .

*Veitchii*, while in its close relative, typical *P. anomala*, they are absent altogether.

In the triploid hybrids, as shown in the table, the expression of this character varies considerably, and no satisfactory explanation for its mode of inheritance was obtained.

*The character of the seedlings.* *Paeonia tenuifolia* differs from all other species of the genus, except *P. Brownii*, in that the cotyledons of the young seedlings are regularly raised above the ground, becoming green and functional as in most dicotyledons, whereas in the other species (except rarely in the subgenus *Moutan*) they are retained within the seed as storage organs, and the first visible leaf of the young seedling is developed from the plumule. In all of the hybrids of which *P. tenuifolia* was the ovulate parent the cotyledons were raised in the seedlings, and this was true also of *P. anomala Veitchii*  $\varnothing$   $\times$  *P. tenuifolia*  $\sigma$  and *Woodwardii*  $\varnothing$   $\times$  *P. tenuifolia*  $\sigma$ . The retention of the cotyledons underground must be considered a specialized characteristic; hence in this as in the previous

cases the more primitive characteristic tends to be dominant in interspecific hybrids.

*The leaf characteristics.* In their leaf characteristics the hybrids are as a rule intermediate between their parents, and there is no apparent tendency for the primitive leaf, the much lobed but not divided type, to be dominant.

#### THE INHERITANCE OF INTERSPECIFIC DIFFERENCES

In interspecific hybridization, the usual result is that the  $F_1$  hybrid is in every respect nearly or quite intermediate between its parents. EAST (1935) after an able summary of the characteristics of the numerous and well known interspecific hybrids in *Nicotiana* has concluded that the form of any organ in a species is the result of the action of a large number of genes working together to create a typical "reaction pattern," and that "all constituents of the pattern are active in modifying the pattern of the hybrids, and the grade of these modifications is little affected by differences in the amount of chromatin contributed" (p. 433).

The interspecific hybrids of *Paeonia* follow, in general, this principle. Intermediate "reaction patterns" similar to those figured by EAST occur regularly in hybrids between the diploid species in the following characteristics: leaf shape, lobing, and dissection; petal size and shape; shape and degree of development of the staminodial disk; and the shape of the follicles and stigmas. Nevertheless, the following exceptions may be noted.

1. In three characteristics, the reaction patterns of diploid hybrids tended to resemble more closely one parent than the other. These characteristics, sepal number and shape, the presence or absence of a branched inflorescence, and the character of the cotyledons in the seedling, are all of phylogenetic significance in the evolution of the genus, and in every case there is a tendency for the more primitive condition to be dominant over the more specialized or advanced. This is most striking in the case of the primitive nature of the cotyledons of the hybrids involving *P. tenuifolia* since here in some cases a gene introduced through the chromosomes of the pollen parent expresses itself at the earliest stage of the germination of the seed. The evidence from *Paeonia*, therefore, points to the fact that in certain characteristics involving the phylogenetic principle of reduction, most of the mutations which produce the difference between species are of the recessive type. A similar situation was found by CLAUSEN (1931) in *Viola*, where such primitive characteristics as the perennial habit are dominant in interspecific hybrids.

2. In one of the diploid-tetraploid crosses, *P. albiflora*  $\times$  *tomentosa*, the evidence points definitely to the fact that the tetraploid contributes more to the "reaction pattern" of the hybrid than the diploid. The greater resemblance to *P. tomentosa* in sepal characteristics has been described

above (table 2, fig. 3) and the resemblance of the  $F_1$  hybrid to *P. tomentosa* in stature and leaf shape has been described by the senior author in a previous paper (SAUNDERS 1933b). There is an even more striking resemblance to *P. tomentosa* in the appearance of the follicles at anthesis, (fig. 3, C-E).

The triploid hybrids of another cross, *P. albiflora*  $\times$  *officinalis* did not show a striking resemblance to their tetraploid parent, but were nearly intermediate. This has been noted above for the sepal characteristics, and by SAUNDERS (1933b) for their stature. An analysis of leaf patterns was not feasible, due to the extreme complexity and variability of this character in *P. officinalis*, but the type of lobing characteristic of *P. albiflora* was very nearly approached in some of the hybrids. The explanation of the different results in these two sets of diploid tetraploid crosses probably lies in the different origin of the tetraploid parent. *P. tomentosa* is taxonomically a more homogeneous, constant species than *P. officinalis*, and the high number of multivalents found at meiosis in this species (to be described in a later paper), indicates that the two diploid genomes that compose its set are closely homologous, that is, it is genetically an autopolyploid. *P. officinalis* is, however, highly polymorphic, has a low proportion of multivalents at meiosis, and the systematic and genetic evidence points to its allotetraploid nature. Furthermore the diploid genomes composing it were probably derived from widely separated diploid species, which differed profoundly in leaf, sepal, and follicle characteristics. In this connection it is perhaps significant that all three of the *Nicotiana* tetraploids referred to by EAST are probably allotetraploids (GOODSPEED 1934), and that at least one, *N. Tabacum*, is derived from two widely different diploid species. The evidence here presented, therefore indicates that while, as EAST maintains, the amount of chromatin contributed by one parent has little effect on the genic pattern of the hybrid, nevertheless the number of duplicated gene pairs contributed by (that is, the amount of autopolyploidy in) one parent is of significance. The same conclusion was reached by ANDERSON (1936) after a comparison of diploid and tetraploid hybrids of *Tradescantia canaliculata* and *T. subaspera*.

#### SOME ABNORMAL CHARACTERISTICS OF THE HYBRIDS

A discussion of the appearance of these hybrids is not complete without an account of the striking abnormalities that have appeared in some of them. These are:

*Abnormal growth of the petals with partial suppression of the stamens.* In the hybrid, *P. triternata Mlokosewitschii*  $\times$  *P. anomala Woodwardii* the young buds are abnormal in their oblong rather than spherical shape. This is due to the premature elongation of the sepals, and the failure of the

petals to develop at a normal rate. The buds often do not open completely, expanding only enough so that the color of the petals may be seen. At this time the petals have reached normal size, being intermediate between those of the parents of this hybrid. The stamens are almost completely suppressed, appearing as strap-like appendages barely 2.5 mm long, as opposed to 10 mm and 12 mm in the two parents (fig. 4C). In the central flower, these rudiments never produce pollen mother cells, but the lateral flowers contain a few scattered, minute anthers in which are formed pollen mother cells, and, after a very irregular meiosis, a scant amount of sterile pollen.

In the hybrid between *Mlokozewitschii* and *P. anomala*, the young buds are elongate like those of *Mlokozewitschii*  $\times$  *Woodwardii*, but they finally

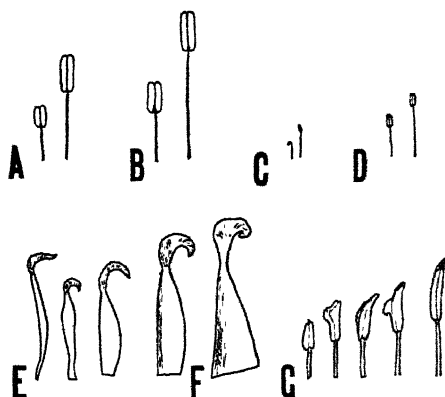


FIGURE 4.—The stamens of: A. *Paeonia anomala* Woodwardii; B. *P. triternata* Mlokozewitschii; C. *Mlokozewitschii*  $\times$  Woodwardii  $\sigma$ ; D. *Mlokozewitschii*  $\times$  *P. anomala* typical  $\sigma$ . All  $\times 2$ . E. Modified stamens of *Paeonia albiflora*  $\times$  *P. anomala* Woodwardii; F. Normal follicle of the same (at anthesis); G. Modified stamens of *P. albiflora*  $\times$  *P. anomala* Veitchii. All  $\times 2$ .

expand into normal flowers. The anthers are somewhat suppressed, being only one sixth to one fifth as long as those of the parents, but they produce some, though a reduced amount, of pollen (fig. 4D).

The third hybrid of this series was a reciprocal to the others, *P. anomala* Veitchii  $\times$  *P. triternata* Mlokozewitschii. It is perfectly normal in bud shape, and in the development of its sepals, petals, and stamens. It produces as much pollen as its parents, although this is almost completely sterile, due to the very abnormal meiosis, which will be described in a subsequent paper. Hence it seems likely that the suppression of growth of the organs appears only when *Mlokozewitschii* is the ovulate parent for this series of crosses.

*Partial sex reversal.* An even more striking abnormality appeared in the crosses between *P. albiflora*, and the various varieties of *P. anomala*. In the hybrids *P. albiflora*  $\times$  *P. anomala* Woodwardii, and *P. albiflora*  $\times$  *P.*

*anomala Beresowskii*, the stamens are replaced by a large number of rudimentary carpels. These have normal looking stigmas, but they are completely open along the ventral suture, and do not produce even the rudiments of ovules (fig. 4E, 4F). The abnormality is most pronounced in the central flower. In the lateral flowers stamens with thin, strap-shaped anthers are often formed, which, in *P. albiflora* × *anomala typica* occasionally produce pollen. The true follicles are normal in appearance, though in some cases more numerous than in normal flowers, and occasionally develop seed. In the final hybrid of this series, *P. albiflora* × *P. anomala Veitchii*, the abnormality is much less pronounced. Here stamens with anthers are regularly produced, but these have pink, sterile tips, and occasionally produce stigmas from their tips or sides (fig. 4G). No reciprocal hybrids of this series have yet been made, so that there is no evidence to indicate whether or not these abnormalities would, as in the previous case, be absent when *P. anomala* and its relatives were used as the ovulate parents.

#### INHIBITION OF GROWTH IN RECIPROCAL HYBRIDS

The abnormalities found in the hybrids between *P. triternata Mlokosewitschii* and the varieties of *P. anomala* may be compared, so far as our results go, with the situation found by many workers in interspecific hybrids of *Epilobium* (LEHMANN 1928, 1931; MICHAELIS 1931 etc.). The parallelism between the two cases may be summarized as follows:

1. In both cases, the abnormalities found in the F<sub>1</sub> hybrids consist exclusively in the inhibition of growth or development in one or more organs or (in the case of *Epilobium*) in the plant as a whole.

2. In both cases there is a reciprocal difference. In *Epilobium* the inhibitions appear most abundantly in the plasma of *E. hirsutum* and *E. parviflorum*, when the pollen parent is a species widely separated taxonomically, while in our case the abnormalities appear under similar conditions in the plasma of *P. triternata Mlokosewitschii*.

3. In both cases the amount of inhibition varies with the genetic constitution of the pollen. SCHWEMMLE (1934) has demonstrated the presence of Mendelian factors in races of *E. roseum* which affect the intensity of inhibition in the plasma of *E. hirsutum*. In *Paeonia*, the inhibition in growth of the anthers is much more marked in *P. triternata Mlokosewitschii* × *P. anomala Woodwardii* than in *Mlokosewitschii* × *P. anomala typica*. In this case there is an interesting correlation between the extent of the inhibition and the luxuriance of the plant. The variety *Woodwardii* has small leaves and flowers; the typical form is taller, and with larger leaves and flowers. It is likely, therefore, that the genic complex in *Woodwardii* which produces the strong growth inhibitions in the plasma of *Mlokosewitschii* is

the same or similar to that which effects the reduction in size of the organs of the homozygote. The stamens of *Woodwardii* are normally smaller than those of the typical form, the average lengths being 9 and 11 mm respectively, but in their hybrids in the plasma of *Mlokošewitschii* the difference is much greater, the average lengths being 2 mm and 7 mm respectively.

The situation in *Paeonia* is also closely parallel to that found by DOBZHANSKY (1935) and DOBZHANSKY and STURTEVANT (1935) in *Drosophila pseudoobscura*. These workers have demonstrated by means of carefully controlled genetical tests that a reciprocal difference in testis size found in crosses between race A and race B of this species is due unquestionably to maternal effect rather than to plasmatic inheritance. In view of their findings, and the similar though less complete results of SCHWEMMLE (1934) in *Epilobium* the writers are inclined to believe that a similar explanation is the most satisfactory one for the phenomena described in *Paeonia*, as well as in *Epilobium*, in spite of a considerable amount of evidence to the contrary in the latter case (MICHAELIS 1933).

As a possible clue to the explanation of this maternal effect the experiments of DELLINGSHAUSEN (1935) which demonstrated a greater viscosity in the cytoplasm of *Epilobium hirsutum* than in that of *E. luteum*, and a correspondingly lower permeability to electrolytes and lipid soluble substances, are worth noting. In the living sporocytes of *Paeonia triternata* and its subspecies the cytoplasm is much more opaque and more heavily granular than that of *P. anomala*, and that of the former species stains more heavily after fixation. This suggests that the cytoplasm of *P. triternata* has, like that of *E. hirsutum*, a high viscosity and low permeability, and that this is directly connected with the maternal effect. Apparently cytoplasm of a high viscosity and a low permeability to lipid soluble substances is most favorable to the action of growth inhibiting genes in the hybrid genome, while a less viscous and more permeable cytoplasm favors the action of the genes determining normal growth.

The case of partial sex reversal parallels that of the inhibition of anther growth in the following respects:

1. Here also the growth of the anthers is inhibited. When not replaced by rudimentary carpels, they are usually thin, strap-shaped structures which produce very little or no pollen.
2. The abnormalities appeared only when *P. anomala* and its subspecies were used as the pollen parents, although the ovulate parent was in this case *P. albiflora*, which is a little less distant taxonomically from *P. anomala* than is *P. triternata Mlokošewitschii* and which does not have the dense appearing cytoplasm of that species.
3. The different varieties of *P. anomala*, when used as pollen parents, produce the same difference in the degree of expression of the character in

the  $F_1$  hybrids. In the  $F_1$  of *P. albiflora* ♀ × *P. anomala Woodwardii* ♂ or *Beresowskii* ♂ (a plant similar in the size of its leaves and flowers to *Woodwardii*) it is most extreme. In *P. albiflora* × *P. anomala typica* it is less so; while in *P. albiflora* × *P. anomala Veitchii*, which has leaves and flowers as large as or larger than those of subsp. *typica*, in the  $F_1$  hybrid it was the least noticeable.

4. In both cases the character is expressed most strongly in the central and less so in the lateral flowers.

In plants, sex reversal can be caused either by the environment (Cannabis, SCHAFFNER 1921), or by the action of combinations of genes (Zea, JONES 1934; Nicotiana, GOODSPEED 1930). Neither of these agencies can explain satisfactorily the present case. The hybrid plants have been growing for several years in the same plot with normal species and hybrids, and have year after year produced unfailingly these abnormalities. Hence the action of the environment can in this case be dismissed without question. On the other hand, no similar sex reversals have appeared in any of the parent species, or more particularly in any of the numerous horticultural varieties of *P. albiflora* which have been grown in this garden, nor in any interspecific hybrids except those mentioned above. Hence any explanation based purely on genic action must assume that there are certain genes and modifiers in *P. albiflora* and *P. anomala* which acting separately have no effect on sex expression, but, acting together in this particular set of hybrids, produced the results observed. Whether this is the correct explanation of these sex reversals, or whether they are due to a cytoplasmic effect similar to that responsible for the inhibitions in the *Mlokozewitschii* × *anomala* hybrids, will depend on the nature of the reciprocal hybrid, when or if it is obtained.

The authors are much indebted to Professor E. B. BABCOCK for helpful advice and criticism and to MRS. KATHERINE D. JENKINS for the execution of the chart, figure 1.

#### SUMMARY

1. Some of the results of 20 years of hybridization work with wild species and cultivated forms of *Paeonia* are described.

2. The degree of compatibility between diploid species is a valuable criterion for delimiting species and subgenera, but gives no information concerning the interrelationships between the different species of a subgenus.

3. Tetraploid species are as a rule completely compatible with each other.

4. Of the diploid species, *P. albiflora* can be crossed relatively easily with any of the tetraploid species, but the others cross with difficulty or

not at all. The cross *P. albiflora* × *tomentosa* is made most easily with the tetraploid as pollen parent, while in the cross *P. albiflora* × *officinalis* there is no reciprocal difference in compatibility.

5. The  $F_1$  hybrids are morphologically intermediate between their parents, and in crosses involving *P. albiflora* or *P. officinalis* show a remarkable degree of variability.

6. In some characteristics, namely, the number and morphology of the sepals, the presence or absence of side buds, and the character of the cotyledons, there is a definite tendency for dominance of those traits which are less specialized, and therefore phylogenetically primitive.

7. In diploid-tetraploid crosses, there is a strong tendency for the dominance of the characteristics of the tetraploid, if the latter is, as indicated by its morphological and cytological characteristics, nearly or completely of autopolyploid origin.

8. In  $F_1$  hybrids between *P. triternata Mlokosewitschii* and the various varieties of *P. anomala*, there is a marked suppression of the anthers when the former is the ovulate parent, but one series of hybrids with it as the pollen parent is normal in this respect. This suggests the presence of a plasmatic inhibition of anther development, which varies in intensity depending on the subspecies of *P. anomala* used.

9. In  $F_1$  hybrids between the various subspecies of *P. anomala* and *P. albiflora*, when the latter is the ovulate parent, varying degrees of sex reversal of the anthers are found, ranging from the presence of stigmatoid tips on the anthers of *P. albiflora* × *anomala Veitchii* to complete replacement of the anthers by rudimentary follicles in *P. albiflora* × *anomala Woodwardii*. Since there is a marked correlation between the degree of effectiveness of the different varieties of *P. anomala* in producing this abnormality when used as pollen parents on *P. albiflora*, and their effectiveness in producing suppression of the anthers when used on *P. triternata Mlokosewitschii*, it is suggested that the two phenomena are similar in origin.

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# CYTOGENETIC STUDIES IN PAEONIA II. THE CYTOLOGY OF THE DIPLOID SPECIES AND HYBRIDS

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Received September 4, 1937

## INTRODUCTION

THE cytological study of the numerous species and hybrids in the SAUNDERS collection has now been carried on intermittently for five years, during which time a considerable amount of data have been accumulated. The present paper aims to present the most significant findings in the diploid species and hybrids, particularly insofar as they help to explain species relationships in this most complex and interesting genus. The somatic chromosomes of *Paeonia* have already been studied by LANGLET (1928), DERMEN (1933), and DARK (1936), and those in meiosis of some species and hybrids by SAX (1932, 1937b, DARK (1936), and the present writer (HICKS and STEBBINS 1934). These studies have revealed not only very favorable chromosomes for a study of chromatid relationships, but also a large amount of structural hybridity. The latter subject is, of course, of prime importance in connection with interspecific relationships and species evolution, and has received particular attention in the present paper.

## MATERIAL AND METHODS

The source of the plants used for this study has been discussed in detail by SAUNDERS and STEBBINS (1938). Fortunately, due to the presence of a large number of wild and little cultivated species gathered together by Dr. SAUNDERS, the difficulty expressed by DARK (1936), that hybridization under cultivation has obscured the relationships of many paeony species, has largely been overcome. As previously mentioned (SAUNDERS and STEBBINS 1938), this difficulty is confined chiefly to the tetraploid species and varieties of Europe. For this reason they have received less attention than the diploids which, with two exceptions (*P. suffruticosa* and *P. albiflora*) represent nearly or quite pure species genetically. As will be seen these two much cultivated species are cytologically indistinguishable from the wild ones.

The somatic chromosomes were obtained mostly from smear preparations, made according to the "Kochmethode" of HEITZ (1926) and two different modifications of it: (1) that of WHITAKER (1934) and (2) the following method, devised by Dr. A. P. SAUNDERS. A bit of tissue (in *Paeonia* young anthers and stigmas are used in preference to root tips,

since they are more easily obtained) is placed on a slide, flooded with 50 percent acetic acid, covered with a cover glass, and boiled gently on a hot plate for 15–20 seconds; by that time the tissue is translucent and soft. The cover glass is then removed, and the slide is dried as completely as possible with absorbent paper. The tissue is then smeared with the edge of another clean slide, flooded with a fixative (LaCour's 2 BD was most frequently used) and fixed for 5–10 minutes. It is then stained with Newton's iodine-gentian violet in the usual manner. This method, though somewhat erratic, produced excellent results in many cases, success depending largely on the completeness with which the tissue could be smeared so that the smear was only one or two layers of cells in thickness. In many cases the chromosomes were spread out flat, as in WHITAKER's method, so that they could easily be measured. Although the boiling in acetic acid damages considerably the outer cells of the tissue, the inner cells are little or not at all affected, so that if the tissue is well smeared these can be stained to show not only the gross morphology but in addition many of the internal details of the chromosomes.

The meiotic chromosomes were studied entirely from smear preparations fixed in LaCour's 2 BD and stained with Newton's iodine-gentian violet.

Drawings were made with a camera lucida at a magnification of 5000, and were reduced to one-third this size in reproduction.

#### THE SOMATIC CHROMOSOMES

The species of *Paeonia* show a striking similarity in both the number and the morphology of their chromosomes. All are either diploid with  $n = 5$ , or tetraploid with  $n = 10$ . Although the numbers of most of them have already been given by LANGLET (1928) and DARK (1936), the following counts are reported here for the first time. Diploid ( $n = 5$ ): *P. Brownii*, *Broteri*, *obovata*. Tetraploid ( $n = 10$ ): *P. corallina*, *corsica*, *tomentosa*. Those previously reported as tetraploid are *P. tenuifolia* "hybrida," *P. officinalis* and relatives, *P. Wittmanniana*, and *P. coriacea*; all of the others are diploid. *P. corallina* and perhaps *P. corsica* contain both diploid and tetraploid forms, but the tetraploid count reported by LANGLET for "*P. obovata alba*" is probably based on the Chinese form of *P. Wittmanniana* (= *P. Willmottiae* Stapf), which is frequently known in cultivation by the above name.

In their morphology, the chromosome sets of all of the diploid species conform to the same karyotype. This is illustrated in figure 1, which shows the haploid complement, drawn from selected chromosomes of the somatic anaphase, of a representative of each of the diploid species. *P. Brownii*, which is taxonomically and genetically remote from all of the other species, is not illustrated, as it forms a problem by itself, which will be dealt

with in a later paper. Its karyotype is, however, quite similar to those of the other species. This karyotype consists of one pair of long chromosomes with median or submedian constrictions, (designated as A), two pairs of shorter chromosomes, also with median or submedian constrictions (B and C), one pair (D) with submedian to subterminal constrictions (arm ratio about 2:1), and often a small satellite on the shorter arm, and the

TABLE I

*The proportional length of the arms of each member of the haploid chromosome set of the diploid species of Paeonia. In each case, the length of the long arm of the E chromosome is taken as 1.*

| SPECIES  | PROPORTIONAL LENGTH OF ARMS |          |          |          |        | MEAN<br>ARM<br>DEFICIENCY<br>PERCENT |
|--|-----------------------------|----------|----------|----------|--------|--------------------------------------|
|  | A                           | B        | C        | D        | E      |                                      |
| <i>P. Delavayi</i>                             | 1.0, .64                    | .84, .76 | .76, .64 | .80, .44 | 1, .20 | 29                                   |
| <i>P. suffruticosa</i>                         | 1.04, .73                   | .85, .73 | .81, .62 | .77, .42 | 1, .15 | 31                                   |
| <i>P. Emodi</i>                                | 1.02, .83                   | .82, .70 | .74, .59 | .85, .44 | 1, .19 | 30                                   |
| <i>P. anomala typica</i>                       | .97, .80                    | .87, .70 | .77, .53 | .97, .47 | 1, .17 | 28                                   |
| <i>Veitchii</i>                                | 1.03, .76                   | .83, .69 | .79, .62 | .97, .52 | 1, .21 | 28                                   |
| <i>Woodwardii</i>                              | 1.04, .79                   | .88, .71 | .83, .67 | .96, .51 | 1, .17 | 27                                   |
| <i>P. albiflora</i>                            | .97, .80                    | .8, .73  | .87, .67 | .97, .50 | 1, .20 | 25                                   |
| <i>P. tenuifolia</i>                           | 1.0, .83                    | .83, .67 | .87, .67 | .9, .47  | 1, .20 | 26                                   |
| <i>P. triternata</i><br><i>Mlokošewitschii</i> | .89, .71                    | .79, .61 | .68, .46 | .82, .39 | 1, .21 | 34                                   |
| <i>P. Broteri</i>                              | .91, .82                    | .79, .67 | .79, .58 | .85, .39 | 1, .18 | 30                                   |
| <i>P. obovata</i>                              | .89, .71                    | .82, .71 | .75, .57 | .86, .39 | 1, .18 | 31                                   |

final pair (E) with subterminal constrictions and a satellite on the shorter arm.

The size of the chromosomes of all the species is nearly the same, as is evidenced by measurements made at meiotic anaphase. Such size differences as appear in the diagrams are due to unavoidable differences in the cells used for drawing, and comparable differences can be found in the different tissues of any plant of *Paeonia*. The same may be said of the size differences in the figures of DARK (1936). The two forms, *P. Beresowskii* (*P. anomala Beresowskii*), and *P. Smouthii* (*P. albiflora* × *tenuifolia*) whose somatic chromosomes appear smaller than the others (his figures 1d and 1f)

have meiotic chromosomes of exactly the same size as those of the other species. This is illustrated for *P. Smouthi* in his figures 11b and c, and in figure 5 of the present paper, while figure 4 of this paper represents a variety of *P. anomala* taxonomically very close to *Beresowskii*, and with chromosomes of just the same size. Hence differences between the complements of the various species, as noted also by EMSWELLER and JONES (1935) in *Allium*, cannot be very well expressed by a recording of the actual length of the chromosome arms. In *Paeonia*, however, each species is characterized by a definite relation of the lengths of the different arms to each other, and this relation can be expressed and compared in the different species if the length of a particular arm in any given set is used as a unit, and the lengths of the other arms of that set are given in terms of that unit. This was done and results are recorded in table 1. These emphasize the general similarity between the complements of the different species, but show also that, even with the slight differences that exist, there is more similarity between subspecies of the same species and closely related species, than between species widely separated taxonomically. For instance, the correspondence between the figures for the three forms of *P. anomala* is very close, and the three species with entire leaflets and without strong midribs on their innermost sepals, namely, *P. triternata Mlokosewitschii*, *P. Broteri*, and *P. obovata* all resemble each other in the relatively smaller size of their D and particularly their A chromosomes. On the other hand, the wide taxonomic difference between *P. Delavayi*, *P. suffruticosa*, and the herbaceous species is not reflected by any comparable difference in their chromosomes, the greatest difference being the marked inequality in the arms of the A chromosome in the shrubby species, particularly *P. Delavayi*. On the basis of these ratios, the "mean arm deficiency," as defined by LEVITZKY (1931), could be calculated, and is recorded in table 1. As expected, there is little difference between the various species in this figure, and here again similar values are correlated with taxonomic similarity of the varieties and species.

The complements of the tetraploid species are more difficult to measure, on account of the greater number of the chromosomes, and their crowding on the cell plate, but such observations as were made indicate that they are quite similar to those of the diploids. The haploid complement of *P. corsica* (fig. 1K) contains the same types as *P. triternata Mlokosewitschii* and *P. obovata*, its near relatives among the diploid species, but each type is represented by two chromosomes. The inequality of the two representatives of each type, particularly the D and E chromosomes, indicate that the form of *P. corsica* in the SAUNDERS collection is an allotetraploid. This indication is supported by the configurations found at meiosis, as will be described in a later paper.

Unfortunately, no satisfactory preparations to illustrate the somatic chromosomes of the other tetraploid species were obtained. The tetraploid varieties of the *P. corallina* complex resemble somewhat in their external morphology *P. corsica*, and their chromosome complement is also similar. That of *P. tomentosa*, another species of this general relationship, is also similar, all these tetraploid species resembling their diploid relatives in the relatively small size of their A chromosomes, which are hardly distinguishable from the B and C pairs. No cytological preparations have been made of *P. coriacea*, *Wittmanniana*, and *Willmottiae*, which are not as yet well established in the SAUNDERS garden. LANGLET'S report of the tetraploid number for these species is supported by observations of meiosis in hybrids of them with *P. albiflora* and *tomentosa*, and by measurements of their stomata.

The complex of *P. officinalis*, the largest and most polymorphic of the species complexes in the genus, deserves special attention on account of other evidence concerning its origin, and will be treated in a later paper.

#### MEIOSIS IN THE DIPLOID SPECIES

HICKS and STEBBINS (1934) described some features of meiosis in *P. albiflora*, *tenuifolia*, *triternata Mlokosewitschii*, and *suffruticosa*, in which emphasis was placed on certain abnormalities: asynapsis, abnormal separation of bivalents, fragmentation, and "chromatid fusion." The latter phenomenon is in most cases associated with fragmentation (HICKS and STEBBINS 1934, figs. 1 and 2), and is therefore the result of chiasma formation in an inverted segment (McCLINTOCK 1933; DARK 1936). Nevertheless, "chromatid fusion" may occasionally occur without any evident fragment (SAX 1932, fig. 12), and in these cases has probably resulted from some other cause. Fragmentation may not infrequently occur from causes other than chiasma formation in an inverted segment, as is clearly shown by HICKS and STEBBINS (1934 fig. 19) and by figures 6A and 6D presented here (see below, under description of hybrids). Of the diploid species which were not studied previously, two, *P. anomala*, particularly the forms *Veitchii* and *Woodwardii*, and *P. Delavayi* are important as parents of the hybrids to be considered here. These resemble most *P. triternata Mlokosewitschii* in their relatively high percentages of fragmentation and of abnormal separation. These are recorded in table 2, which includes also another plant of *Mlokosewitschii*, whose behavior was somewhat different from that recorded in the previous paper, and three additional strains of *P. albiflora*. The plant called "Vilmorin" is a white single, received from Vilmorin et Cie., Paris, as the wild *P. albiflora*, although it differs considerably from any herbarium specimens of this form seen by the writer. It has larger relatively narrower leaves and larger flowers, with broader,

TABLE 2  
Frequency of abnormalities in the species.

|   | PERCENT OF CELLS<br>WITH<br>UNIVALENTS | PERCENT OF CELLS<br>WITH<br>FRAGMENTS | PERCENT OF CELLS<br>WITH ABNORMAL<br>SEPARATION |
|---|--|---------------------------------------|---|
| <i>P. albiflora</i>                                       |  |                                       |   |
| "Vilmorin"  | 13                                     | 3.8                                   | 2.5   |
| Saunders no. 725  | 9.8                                    | 5.3                                   | 1.8   |
| Saunders no. 1505   | 2.2                                    | 4.2                                   | 0.4   |
| <i>P. anomala</i>   |  |                                       |   |
| <i>Veitchii</i>   | 21                                     | 19                                    | 3.5   |
| "Hesse"   | 26                                     | 29                                    | 6.7   |
| <i>P. Emodi</i>   | 18                                     | 7.2                                   | 0.7   |
| <i>P. triternata</i> <i>Mlokosewitschii</i> ,<br>No. 2792 | 22                                     | 19                                    | 1.5   |
| <i>P. Delavayi</i><br>var. <i>lutea</i>                   | 33                                     | 18                                    | 7.6   |

less strongly notched petals. No. 1505 is a white single seedling from an ordinary horticultural variety.

From table 2 it is evident that the species fall into two groups with respect to the frequency of abnormalities. *P. albiflora*, *Emodi*, *tenuifolia*,

TABLE 3  
Chiasma frequencies of the species.

|   | NUMBER<br>OF NUCLEI | $\bar{X}$ <i>ta</i><br>BIVALENT<br>S.E. | TERMINAL $\bar{X}$ <i>ta</i><br>BIVALENT | TERMINALIZA-<br>TION<br>COEFFICIENT |
|---|---------------------|---|--|-------------------------------------|
| <i>P. albiflora</i>                         |                     |   |  |                                     |
| "Vilmorin"                                  | 50                  | 1.59 ± 0.04                             | 0.71                                     | 0.44                                |
| "Silvia Saunders"                           | 75                  | 1.75 ± 0.03                             | 0.83                                     | 0.47                                |
| Saunders no. 725                            | 20                  | 1.60 ± 0.06                             | 0.68                                     | 0.42                                |
| Saunders no. 1505                           | 50                  | 1.72 ± 0.04                             | 0.93                                     | 0.54                                |
| <i>P. anomala</i>                           |                     |   |  |                                     |
| <i>Veitchii</i>                             | 30                  | 1.93 ± 0.06                             | 1.16                                     | 0.45                                |
| <i>Woodwardii</i>                           | 30                  | 1.75 ± 0.05                             | 0.92                                     | 0.52                                |
| "alba" (Hesse)                              | 30                  | 1.85 ± 0.07                             | 0.77                                     | 0.42                                |
| <i>P. Emodi</i>                             | 30                  | 1.70 ± 0.08                             | 0.89                                     | 0.52                                |
| <i>P. tenuifolia</i>                        | 30                  | 1.85 ± 0.05                             | 0.99                                     | 0.54                                |
| <i>P. triternata</i> <i>Mlokosewitschii</i> | 34                  | 1.85 ± 0.06                             | 0.86                                     | 0.46                                |
| <i>P. Delavayi</i> var. <i>lutea</i>        | 32                  | 1.80 ± 0.06                             | 0.92                                     | 0.50                                |
| <i>P. suffruticosa</i>                      | 30                  | 1.58 ± 0.06                             | 0.62                                     | 0.41                                |

and *suffruticosa* have a relatively low percentage of all types, while in *P. anomala*, *triternata*, and *Delavayi* this is relatively high. Fresh preparations of the only clone of *P. tenuifolia* available, and of *P. albiflora* "Silvia Saunders" showed conditions essentially similar to those already recorded, as did also those of another plant of *P. suffruticosa*.

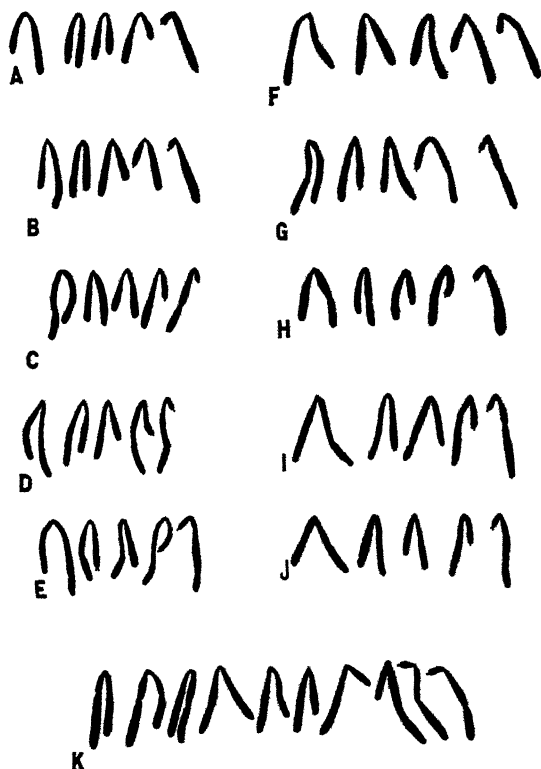


FIGURE 1.—The haploid chromosome sets, drawn from somatic anaphases of: A—*P. Delavayi*; B—*P. suffruticosa*; C—*P. Emodi*; D—*P. anomala typica*; E—*P. anomala Veitchii*; F—*P. albiflora* (Vilmorin seedling); G—*P. tenuifolia*; H—*P. triternata Mlokošewitschii*; I—*P. Broteri*; J—*P. obovata*; K—*P. corsica* (Barr's var.).

In addition, the chiasma frequency at metaphase of the various species was computed, and is shown in table 3. The most striking fact evident from a study of these figures is the great similarity between the species, and the lack of any correlation between chiasma frequency and percentage of asynapsis. This is due to the higher percentage of bivalents with three and four chiasmata in *P. anomala*, *triternata Mlokošewitschii*, and *Delavayi*, as is illustrated in figure 2, showing the five bivalents from a nucleus of *P. anomala Veitchii* with a relatively high number (12) of chiasmata. There is a consequently greater coefficient of variability of the mean chiasma fre-



quency in these species, though this difference is only in some cases significant, for example, as between *P. triternata Mlokoewitschii*, C.V. =  $45.7 \pm 2.5$ , and *P. tenuifolia* C.V. =  $37.2 \pm 2.1$  (D/S.E. = 2.6). This difference is illustrated in the diagram, figure 3.

The terminalization coefficients at metaphase are essentially similar in all of the species, and here again there is no correlation with the meiotic abnormalities.

In addition to the abnormalities described above, multivalents have been found in two diploid species. In *P. Delavayi* these are as described and illustrated by DARK (1936), and were interpreted by him as the result of the pairing of reduplicated segments of non-homologous chromosomes.

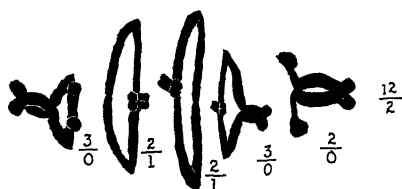


FIGURE 2.—The five bivalents from a nucleus of *Paeonia anomala Veitchii* at first metaphase, showing an unusually high number (12) of chiasmata for this species.

Their occurrence in this species, however, is very rare, as in the material investigated by the writer only two cases were seen in about 200 cells, and with the exception of a doubtful case in *P. suffruticosa*, multivalents of this type have not been found in any other diploid species of *Paeonia*.

In one form of *P. anomala*, including all of the plants received from DR. HESSE (cf. SAUNDERS and STEBBINS 1938) a different type of multivalent was observed in 60–80 percent of the metaphase nuclei. There were either trivalents or quadrivalents, and they were in the form of rings or chains, with or without terminalization of chiasmata (fig. 4A, C, D). They always included the submedianally constricted (D) pair and one of the smaller medianally constricted (B or C) pairs of chromosomes. In most of the nuclei of this form which did not contain multivalents, there were three pairs of equal homologues and two unequal pairs, each involving one B or C and one D chromosome (fig. 4B). This condition apparently resulted from the failure of chiasma formation in two of the arms of an x-shaped multivalent pachytene configuration such as that illustrated by COOPER and BRINK (1931) for *Zea*, and, along with the lack of triple chiasmata in the multivalents, is strong evidence that this race of *P. anomala* is heterozygous for an interchange of non-homologous segments, involving a large portion of the long arm of a D, and of one arm of a B or C chromosome. Two different plants were studied, one from the group received as *P. anomala*, and one from those designated *P. anomala alba*, and since both

showed the same type of multivalent and unequal bivalent configurations, it is very likely that these occur in all of the plants. An extraordinary fact is that their pollen is about 75–80 percent viable, as determined by germination tests. Since cytological observation indicates that in three-fourths of the cells the separation is “disjunctional,” that is, homologous chromosomes pass to opposite poles, this fertility is not unexpected.

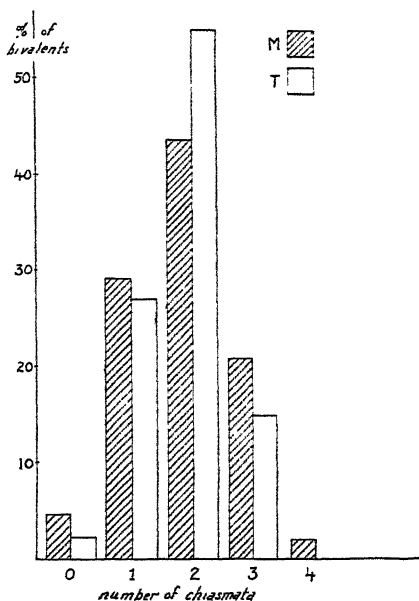


FIGURE 3.—Chiasma distribution in *Paeonia triternata* Mlokosewitschii (at left) and *P. tenuifolia* (at right, not cross-hatched).

An explanation for the preponderance of disjunctional separations in organisms with terminalized chiasmata is given by DARLINGTON (1937, pp. 151–152), that is, that such rings are flexible enough that the “forces of repulsion associated with the centromere are able to effect regular disjunction. . . .” This explanation holds at least to some extent in *P. anomala* even though the chiasmata are not completely terminalized, since they are mostly near the distal ends of the chromosome in this configuration, and are never numerous as in *Pisum* and *Hyacinthus*. Furthermore, the chromosomes of *Paeonia* are unusually large for their nucleus, giving a “crowded” cell plate (DARLINGTON 1936a), in which the free distal ends of the longer bivalents are actually near the end wall of the cells. Hence it seems likely that pressure from the cell walls would in most cases prevent the formation of the open non-disjunctional type of configuration illustrated in figure 4D, and would absolutely prevent the linear orientation of a trivalent. This latter configuration has not been found.

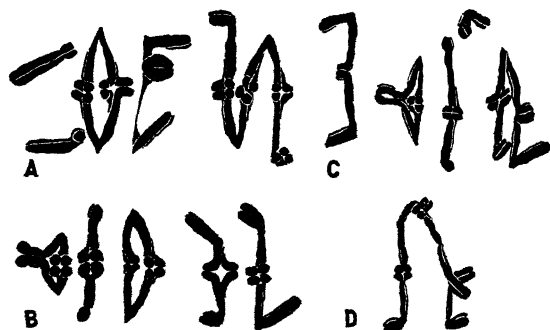


FIGURE 4.—A. Chromosome complement from a nucleus at first metaphase of *P. anomala* (Hesse seedlings), showing a chain quadrivalent. B. Another nucleus, showing two unequal bivalents. C. Another nucleus, showing a trivalent and a univalent. D. A single quadrivalent, arranged "non-disjunctionally."

TABLE 4  
Characteristics of the hybrids

|  | AVERAGE NO.<br>UNIVALENTS<br>PER NUCLEUS | PERCENT<br>OF CELLS<br>WITH<br>MULTIVALENTS | PERCENT<br>OF INVERSION<br>CONFIGURA-<br>TIONS | <i>X ta</i><br>BIVALENT | TERM.<br>COEFF. | PERCENT<br>OF<br>STERILE<br>POLLEN |
|--|--|---|--|-------------------------|-----------------|------------------------------------|
| <i>P. albiflora</i> × <i>tenuifolia</i>  |  |   |  |                         |                 |                                    |
| 8280   | 1.26 ± 0.15                              | 0   | 2.7 ± 0.4                                      | 1.21                    | 0.47            | 50-60                              |
| 8276   | 0.92 ± 0.11                              | 0   | 2.2 ± 0.4                                      | 1.41                    | 0.42            | 50-60                              |
| 6556   |  | 0   |  | 1.39                    | 0.44            | 50-60                              |
| <i>P. albiflora</i> × <i>anomala Veitchii</i> 6428   | 1.14 ± 0.16                              | 2.7 ± 1.7                                   | 4.4 ± 1.6                                      | 1.29                    | 0.43            | 95-96                              |
| <i>P. anomala Beresowskii</i> × <i>Emodi</i>   | 2.62 ± 0.16                              | 2.4 ± 1.7                                   | 16.0 ± 3.2                                     | 0.95                    | 0.36            | 95-99                              |
| <i>P. tenuifolia</i> × <i>anomala Veitchii</i> ,<br>no. 6430                               | 3.56 ± 0.18                              | 20 ± 4                                      | 11.9 ± 1.8                                     | 0.82                    | 0.36            | 96-99                              |
| <i>P. tenuifolia</i> × <i>anomala Woodwardii</i> ,<br>no. 5093                             | 2.74 ± 0.16                              | 9.3 ± 2.8                                   | 6.3 ± 1.3                                      | 0.96                    | 0.47            | 96-99                              |
| <i>P. tenuifolia</i> × <i>triternata Mlokose-<br/>witschii</i>                             | 3.44 ± 0.17                              | 15 ± 3.6                                    | 7.9 ± 1.5                                      | 0.78                    | 0.49            | 96-99                              |
| * <i>P. tenuifolia</i> × <i>triternata</i> ? no. 6581                                      | 3.46 ± 0.19                              | 8 ± 2.7                                     | 7.4 ± 1.4                                      | 0.77                    | 0.39            | 96-99                              |
| <i>P. anomala Veitchii</i> × <i>triternata</i><br><i>Mlokosewitschii</i> , nos. 3468, 3469 | 3.81 ± 0.16                              | 16.2 ± 3.6                                  | 13.3 ± 2.3                                     | 0.77                    | 0.50            | 96-99                              |
| no. 5360   | 2.61 ± 0.22                              | 21.4 ± 5.4                                  | 7.8 ± 1.4                                      | 0.94                    | 0.38            | 96-99                              |
| <i>P. Delavayi</i> var. <i>lutea</i> × <i>suffruticosa</i> ,<br>"Argosy"                   | 3.66 ± 0.22                              | 15.1 ± 2.6                                  | 11.2 ± 1.8                                     | 0.84                    | 0.45            |                                    |

\* This is the plant received from Barr and Sons as *P. anomala*, and is discussed elsewhere (STEBBINS 1938).

#### MEIOTIC BEHAVIOR IN THE DIPLOID HYBRIDS

The group of hybrids which are the subject of this study includes all possible crosses between four of the herbaceous species of the subgenus Paeon, *P. albiflora*, *P. anomala Veitchii* and *Woodwardii*, *P. tenuifolia*, and *P. triternata Mlokosewitschii*, and between the two shrubby species of the subgenus Moutan, *P. Delavayi* and *P. suffruticosa*. Members of different subgenera cannot be crossed, and within Paeon the cross *P. albiflora* × *triternata Mlokosewitschii* has failed in spite of repeated efforts (SAUNDERS and STEBBINS 1938).

The characteristics of meiosis in the various hybrids are summarized in

table 4. Since in these hybrids many nuclei contain only 3, 2, 1, or even 0 bivalents, the mere listing of the percentage of cells with univalents is not significant as it is in the species, where practically all of the nuclei contain either 5 or 4 bivalents. The average number of bivalents per nucleus, moreover, cannot be accurately determined in many of the hybrids, on account of the frequent presence of trivalent and quadrivalent associations. Hence the amount of chromosome pairing characteristic of each hybrid is best expressed by giving the average number of univalents per nucleus. The percentage of cells with fragments also cannot be satisfactorily listed, since in many cases the univalents split at first anaphase, producing single chromatids which are in many anaphases impossible to distinguish from large single chromatid fragments. On the other hand, the chiasma frequency is here low enough so that two chiasmata occur only rarely in an arm bearing an inverted segment, and hence the percentage of bivalents showing a fragment and a continuous chromatid is some indication of the amount of pairing of inverted segments in these hybrids.

This table shows that the hybrids are of two types. In the first type, represented by *P. albiflora*  $\times$  *tenuifolia* and *P. albiflora*  $\times$  *anomala Veitchii*, the chromosome pairing is nearly as complete as in some of the species, and the reduction in chiasma frequency is relatively slight. Furthermore, the percentage of nuclei bearing fragments, though greater than that in the parents of these two hybrids (HICKS and STEBBINS 1934, table 1) is about the same as in several of the diploid species. There is a low percentage of configurations indicating chiasma formation in an inverted segment, and multivalent configurations, as in the species, are absent or rare. The terminalization coefficient in all of the hybrids shows the same range of variation as in the species, and since the prophase stages could not be studied in this material, this figure is of relatively little significance in *Paeonia*. Figure 5 shows a first metaphase nucleus of *P. albiflora*  $\times$  *tenuifolia* no. 8276, in which there is complete pairing, a relatively high number of chiasmata (9), and a case of distal interlocking, which is not uncommon in *Paeonia*. It is interesting to note that in their cytological as well as their morphological characteristics the seedlings of this cross produced recently in the SAUNDERS garden, represented in this study by nos. 8276 and 8280, are essentially the same as the clone, "*P. Smouthii*," of the same hybrid which was produced almost a hundred years ago, and is represented in this work by three different plants, no. 6556 recorded here, and nos. 6550 and 6582, recorded by HICKS and STEBBINS (1934). Since this is true in spite of the fact that the slides on which the studies are based were made during three different seasons, 1932, 1933, and 1934, there is good evidence that normal fluctuations in the external environment have little effect on meiosis in this hybrid.

In regard to pollen sterility nos. 8276 and 8280 also compare rather closely with *P. Smouthi*. Although there are about 40 percent of normal appearing grains, only a small percentage of these are viable under artificial conditions of germination. In the other hybrid of this group, *P. albiflora* × *anomala Veitchii*, nearly all of the pollen is shrunken and obviously sterile.

The members of the second group, which constitute the majority of the diploid hybrids, show a much higher degree of meiotic abnormality in every respect. First metaphase nuclei which show complete pairing are relatively rare, occurring in only 6–13 percent of the total while the most frequent number of bivalents per nucleus is correspondingly lower, and in every case the number of chiasmata per (potential) bivalent is less than

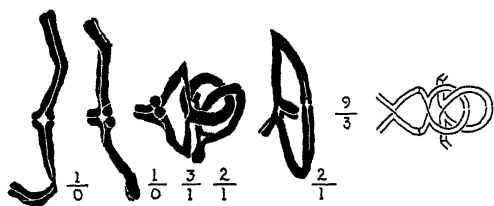


FIGURE 5.—The five bivalents at first metaphase from the hybrid *P. albiflora* × *tenuifolia* (no. 8276), showing an unusually large number of chiasmata for this hybrid, and a case of distal interlocking.

one. The hybrids are all essentially the same in this respect, and such significant differences as exist occur between hybrids whose parents were different plants of the same species, that is, between *P. tenuifolia* × *P. anomala Woodwardii*, as well as between the two numbers of *P. anomala Veitchii* × *P. triternata Mlokošewitschii*, nos. 3469 and 5360, of which the *Mlokošewitschii* parents were different plants.

Fragmentation is extremely common in these hybrids and may result either from chiasma formation in an inverted segment, or from other unknown causes. Both types are shown for *P. Delavayi* var. *lutea* × *P. suffruticosa*, "Argosy," in figure 6A in which the left hand bivalent shows a fragment still paired with the normal chromatids, while one of the univalents has split, and of the separate chromatids one has broken in two, and the other, situated near the upper pole of the spindle, shows a partial break. In figures 6B–D, also from "Argosy," fragmentation of bivalents is seen. Figure 6B shows a stage preliminary to that illustrated by HICKS and STEBBINS (1934 fig. 19), while figures 6C and 6D show two stages of the same type of fragmentation involving two chromatids.

An abnormality not encountered in any of the species or in the hybrid *P. albiflora* × *tenuifolia*, but occurring in all of the other diploid hybrids is the formation of "restitution nuclei" at the end of the first division of

meiosis, and the consequent presence of a single metaphase plate at the second division, resulting in the formation of dyads of diploid gametes. A second metaphase plate of this type is illustrated in figure 7 of *P. anomala Veitchii* × *P. triternata Mlokosewitschii*. While generally most frequent in the hybrids in which chromatin bridges are also the most commonly found, and due partly to the persistence of these bridges, as is evidenced by the frequent presence of dumbbell shaped nuclei, the formation of restitution nuclei at first telophase occurs in one hybrid in which chromatin bridges are relatively uncommon, *P. albiflora* × *anomala Veitchii*. This hybrid, although it forms only two percent more chromatin bridges than *P. albiflora* × *tenuifolia*, nevertheless produces restitution

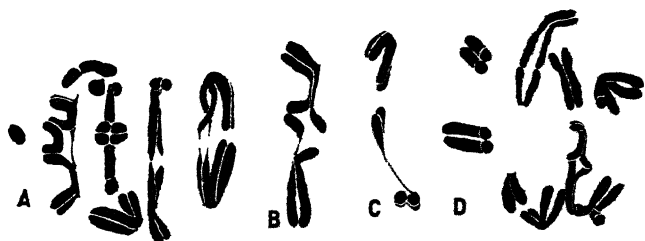


FIGURE 6 A-D.—Four different types of fragmentation in *P. Delavayi* var. *lutea* × *suffruticosa* "Argosy." Explanation in the text.

nuclei in 12 percent of its sporocytes, while no such nuclei have been seen among about 300 sporocytes at second metaphase in *P. albiflora* × *tenuifolia*. On the other hand, *P. anomala Veitchii* × *tenuifolia*, although it has almost three times as high a percentage of chromatin bridges (11.9 percent) as *P. albiflora* × *anomala Veitchii* nevertheless forms only 14 percent of restitution nuclei. That persistent chromatin bridges do not in most cases produce by themselves restitution nuclei is made evident by the frequent presence in such species as *P. suffruticosa* and *P. triternata Mlokosewitschii* (HICKS and STEBBINS 1934, figs. 2 and 8) of quite distinct homoeotypic metaphase plates connected by such a bridge.

The most characteristic and unusual meiotic abnormality in the hybrids of this group except for *P. anomala Beresowskii* × *Emodi* is the formation of trivalent, quadrivalent, and quinquevalent associations. These configurations often show at late metaphase or early anaphase triple or quadruple chiasmata, like those illustrated by DARK (1936, fig. 7) for *P. Delavayi*. This suggests that they are probably produced by the pairing of reduplicated segments, as suggested by DARK, but another explanation is possible, that many small interstitial translocated segments are present in these hybrids. This explanation however, is made less probable than DARK's by the following facts:

1. Study of a single chromosome pair (that with subterminal constrictions) in different cells of *P. Delavayi* var. *lutea*  $\times$  *P. suffruticosa* indicates that chiasmata may form at almost any point along the length of the chromosomes of this pair (fig. 8, A-G). The separation of a completely

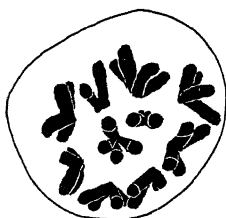


FIGURE 7.—A "restitution nucleus" at second metaphase (polar view) of *P. anomala* *Veitchii*  $\times$  *triternata* *Mlokosewitschii*.

terminal chiasma is not included in this figure, but is illustrated in figure 11A, and many such chiasmata were observed at metaphase in other cells. Nevertheless, at least three different types of multivalent configurations occur which involve one or both members of this pair and those of a different pair (figs. 10A, E, F, G, H). If these are due to translocations, therefore, the total length of each translocated segment cannot be greater than  $1/10$  the length of the chromosome.

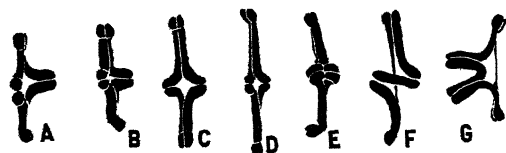


FIGURE 8.—The E bivalent of *P. Delavayi* var. *lutea*  $\times$  *suffruticosa*, from 7 different nuclei, showing chiasmata in normal (A-E) and in two different inverted segments (F, G).

2. The low chiasma frequency characteristic of all of the hybrids in which these multivalents are abundant indicates a lack of homology between the parental chromosomes, and a relatively loose association between them at pachytene. The formation of triple and quadruple chiasmata, however, requires a rather intimate type of pairing in certain regions, with frequent exchanges of partners as in polyploids (DARLINGTON 1937, pp. 119-122), which could be expected to occur only rarely in such hybrids. On the other hand, reduplicated segments occurring in two different chromosomes derived from the same parent would be completely homologous, and hence might be expected to pair rather frequently, if the corresponding segments derived from the other parent were less homologous. Further evidence in this direction is presented below.

3. In one case (fig. 9, A-C), two different cells of the hybrid *P. tenuifolia*  $\times$  *P. anomala* Veitchii each contained a trivalent apparently involving the same three chromosomes, one with a submedian and two with median constrictions. In both figures two chiasmata are present, one normal, involving the two median chromosomes, and the other, formed in an inverted segment, involving one median and one submedian (D) chromosome. The former type of chiasma is in figure 9A in a different arm from the latter, while in figure 9B the two chiasmata are in the same arm. The inverted chiasma is in figure 9A nearer the distal end of the median and the proxi-



FIGURE 9.—A, B. The same trivalent from two different nuclei of *P. tenuifolia*  $\times$  *anomala* Veitchii; C, a bivalent from another nucleus involving the two D chromosomes; D and E two different types of trivalents from the same hybrid. Further explanation in the text.

mal end of the submedian chromosome, while in figure 9B it is nearer to the distal end of the submedian chromosome. If, as is suggested by the similar size of the two fragments, two chiasmata were formed at different points along the same inverted pairing segment, then the two members of the submedian (D) pair should not form chiasmata in this segment, unless the segment occurs in all three chromosomes. The fact that in another cell of the same hybrid the two D chromosomes were found paired in this fashion (fig. 9C) indicates that the two trivalents illustrated result from chiasma formation in an inverted reduplication.

#### ANALYSIS OF STRUCTURAL HYBRIDITY IN *P. Delavayi* VAR. *lutea* $\times$ *P. suffruticosa*

On account of their variety and complexity, configurations of this type have been studied intensively in one hybrid, *P. Delavayi*  $\times$  *suffruticosa*, "Argosy," of which exceptionally good preparations were obtained during the seasons of both 1933 and 1934. The two sets of preparations show no observable differences from each other, suggesting that even this very irregular type of hybrid meiosis is in *Paeonia* little affected by normal fluctuations in the external environment.

The simplest type of configuration found is illustrated in figure 10A, a quadrivalent resulting from pairing of a reduplicated segment involving the distal end of the long arm of the subterminally constricted (E) pair and of one of the medianally constricted (A, B, or C) pairs. The reduplication in figure 10B involves the shorter arm of a submedianally constricted (D) chromosome and a proximal segment of a median pair.



In all of the other multivalents illustrated chiasma formation has taken place in inverted as well as reduplicated segments. In figure 10C two submedian (D) homologues are held together by a chiasma in an inverted proximal segment of their long arms, and the distal end of each is paired with a medially constricted chromosome, in one case by a reduplicated segment which is apparently at the very end of one, but not of the other

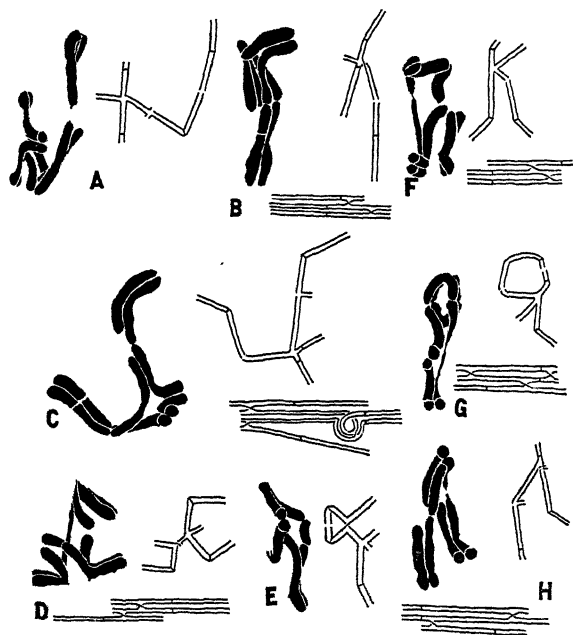


FIGURE 10.—Multivalent configurations from different nuclei of *P. Delavayi* var. *lutea*  $\times$  *suf-fruticosa* "Argosy," showing with each configuration a diagrammatic interpretation of the metaphase configuration and the probable pachytene configuration from which it arose. Further explanation in the text.

chromosome. In all of the other configurations illustrated a chiasma has formed in an inverted reduplication of otherwise non-homologous chromosomes, while one or both of these have in addition formed a normal chiasma with their true homologue. In figures 10D and 10E the inverted reduplication is distal to the normal chiasma, so that the fragment is paired with chromatids representing only two of the three members of the trivalent, while in figures 10F, 10G, and 10H, it is proximal, and the chromatid fragment is paired with chromatids of three different chromosomes. Figure 10H shows at the left evidence of a deficiency in one of two homologues. In figure 10B a single chromatid has broken, while figure 10C shows a type of fragmentation similar to that in figure 6D. Figure 11A shows a whole nucleus in which half of the ten chromosomes have formed a quinquevalent which involves the pairing of three different non-homol-

ogous chromosomes by means of reduplicated segments. Figure 11B is a very complex trivalent containing two fragments, one larger and one smaller. It has apparently resulted from chiasma formation in two different inverted segments, one belonging to homologous chromosomes, and the other an inverted reduplication.

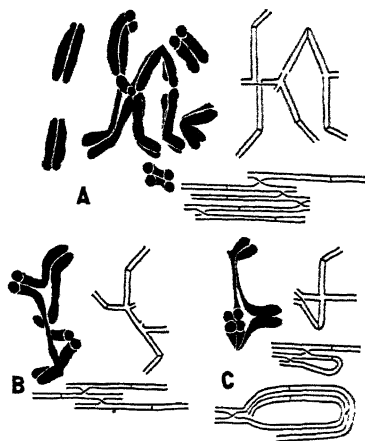


FIGURE 11.—Further configurations from "Argosy." Explanation in the text.

Figure 11C is a remarkable type of bivalent found only occasionally, for which two possible explanations exist. Either it is the result of a reduplication involving the two ends of the same chromosome, or of a long inversion involving the attachment constriction. The latter, suggested to the author by Dr. D. G. CATCHESIDE, is the more likely.

#### DISCUSSION

##### *The significance of interspecific differences in the character of meiosis*

The diploid species of the Old World subgenera of *Paeonia* are all essentially alike in the size and external morphology of their somatic chromosomes, and the present work demonstrates also their slight variability in two characteristics of meiosis, the frequency and distribution of the chiasmata at metaphase. Nevertheless, there are definite differences between the species in one cytological characteristic—the percentage of meiotic abnormalities, particularly of fragmentation. In this respect the species can be roughly divided into two groups: *P. albiflora*, *Emodi*, *tenuifolia*, and *suffruticosa* with a low percentage of these abnormalities, and *P. anomala*, *triternata*, and *Delavayi* in which they are relatively frequent. As emphasized previously (HICKS and STEBBINS 1934) there is no correlation between the percentage of these abnormalities and that of

asynapsis or of pollen sterility, and from the results of this study it may be said that there is also none between it and the chiasma frequency. In fact the chiasma frequencies of those species with frequent abnormalities have slightly, though not significantly higher averages than those in which they are less common.

Since the fragmentation is due chiefly to chiasma formation in inverted segments, and since the chiasma frequency of all of the species is approximately the same, the percentage of fragmentation in a species probably is roughly proportional to that of the inverted segments for which it is heterozygous. Therefore *P. triternata*, *anomala*, and *Delavayi*, or at least the clones of these species here studied, are heterozygous for a considerable number of inversions. The following points favor the view that in these species there is a relatively large number of small interstitial inverted segments, rather than that the segments are of the same number as, but larger than those in the species which show few chiasmata in inverted segments:

1. Occasional configurations resulting from chiasma formation proximal to an inversion, as well as in it, were found (figs. 10D, E).

2. In some cells both arms of all of the medianally and submedianally constricted chromosomes were united by chiasmata, but in none of these was there a chromatin bridge and fragment. In such cases chiasma formation must have been in every case distal to the inverted segments.

3. No configurations were found corresponding to those illustrated in RICHARDSON'S (1936) Diagram 1 as resulting from the formation of two chiasmata within an inverted segment.

4. In one hybrid, *P. Delavayi* var. *lutea* × *P. suffruticosa*, the length of one inversion was determined as not exceeding 1/10 the length of the metaphase chromosome (fig. 8). Probably similar short inversions are responsible for the rare occurrences of the bridge fragment configurations in such species as *P. albiflora*.

It will be noted that the three species with more numerous inversions not only represent both of the Eurasian subgenera (*P. Delavayi* in subgenus Moutan, *P. anomala* and *P. triternata* in subgenus Paeon), but that the last two species are, respectively, nearly the most primitive and nearly the most advanced of their subgenera (STEBBINS 1938). Furthermore, they represent the geographical extremes of the range of the genus, since the range of *P. triternata*, in the region about the Black Sea, is near the southern limit of the range of at least the diploid species, while *P. anomala* ranges much farther north than any other species of *Paeonia*. There is, however, a significant taxonomic characteristic which these species have in common. All three of them are morphologically very variable in nature, while the other species, *P. albiflora*, *Emodi*, *suffruticosa*, and *tenuifolia* are

much more constant in the wild state, although in *albiflora* and *suffruticosa* there is a multitude of horticultural forms. This suggests that, although the production of new varieties under cultivation has taken place chiefly through gene mutation, the variations found in nature are due at least partly to the formation of  $\frac{1}{2}$  inversions. The suggestion of HICKS and STEBBINS (1934), that the production of varying forms, or ecotypes, might be effected by the accumulation of the smaller, non-lethal deficiencies produced by fragmentation is not substantiated by a study of the somatic chromosomes of the different forms, since no significant difference is found between the different forms of a species. On the other hand, MÜNTZING (1934) has demonstrated that new types of chromosomes could arise through breakage of chromatin bridges, some of which may have translocations added, so that by alternation of translocation and fragmentation considerable structural change could be produced without visible alteration of the karyotype. However, one crucial test of this hypothesis as applied to these species of *Paeonia* does not favor it. Although one would expect, on this basis, that hybrids between two of the races of any of these species would show more structural differences than the races themselves, this has not proved to be the case. A cursory examination of meiosis in *P. anomala* *Veitchii*  $\times$  *Woodwardii* was sufficient to determine that it is as regular, if not more regular than in the parent species, and the hybrid is as fertile as its parents. For this reason, furthermore, the role of structural change in producing isolation, and thus favoring independent mutation, as mentioned by STERN (1936), cannot have been important in the evolution of the races (ecotypes) of *P. anomala*.

DARLINGTON (1936b, 1937, p. 273) has given an explanation which exactly fits the case of these species of *Paeonia*. He points out that the presence of many small inversions, by inhibiting effective crossing over in the regions in which they occur, and thereby "holding groups of genes together as units" may establish a "group-discontinuity, a fission in the species." This effect is exactly what is seen in the taxonomic characteristics of *P. Delavayi*, *anomala*, and *triternata*. In *Paeonia*, with its low number of chromosomes, crossover suppression would be unusually effective in producing discontinuity.

#### *Differences in the frequency and distribution of chiasmata*

Although the chiasma frequencies and terminalization coefficients at metaphase do not show any differences between the species that are correlated with any other characteristics, there are nevertheless some interesting points to be noted. In the first place, the chiasma frequencies of both species and hybrids are all about 0.3–0.4 higher than those recorded for the same forms by DARK (1936). Dr. DARK has very kindly examined repro-

ductions of figures 3 and 5, and assures me that this difference is actual. Two possible explanations for this occur to me:

1. The strains in the SAUNDERS garden are different from those examined by DARK. This seems hardly likely in view of the consistency of the differences. Furthermore one form, *P. Smouthii*, is a sterile hybrid which is very difficult to produce, and therefore every probability exists that the clone of this species in Mr. STERN's garden is identical with that of Dr. SAUNDERS.

2. The differences in climatic conditions between England and central New York are responsible. During the early part of the growing season at Clinton, from late April until mid-May, sudden changes of temperature are the rule. The nightly temperature may drop to 30° F (−1° C) or even 20° F (−7° C) as on May 15, 1936, while the daytime temperatures usually reach 75–80° F (24–27° C) for a few days during that period. The extremes over the same period in England are much less. Although SAX (1937a) has shown that sudden changes of temperature produce asynapsis in *Tradescantia* and *Rhoeo*, this need not be true in all genera, particularly those whose natural distribution and time of flowering is that of *Paeonia*. All of the species whose chiasma frequency is here reported are natives of regions where extremes of temperature are the rule (Siberia, Manchuria, western China, the Himalaya and Caucasus Mountains) and are undoubtedly adjusted to them. Hence the climate of Central New York is normal for them, while that of England is not. This abnormality of the external environment may be responsible for the unusually low chiasma frequency and high percentage of asynapsis found by DARK. A crucial test of this explanation would, of course be obtained by keeping a plant of *Paeonia* in a constant temperature chamber during the beginning of its spring growth.

#### *Distribution of abnormalities in the hybrids*

Although no type of meiotic abnormality occurs in the hybrids which is not found also in at least some of the species, all of the abnormalities are, as would be expected, more common in the hybrids than in their parents. An unexpected result, however, is that the amount of abnormality in the hybrids is not at all proportional to the difficulty with which their parents can be crossed, or with the degree of taxonomic difference between them. The easiest cross to make between diploid species is *P. tenuifolia* × *triternata* *Mlokosewitschii*, but the resulting hybrid has a very abnormal meiosis. On the other hand, the two most difficult crosses to make are *P. albiflora* × *tenuifolia* and *P. albiflora* × *anomala*, and yet these two hybrids are in their meiotic behavior the least abnormal of the series. The taxonomic differences will be discussed below in relation to the problem of chromosome pairing, but it may be mentioned here that, if one considers the sum

total of vegetative and floral characteristics, *P. albiflora* is farther from *P. tenuifolia* than is any other species of the subgenus *Paeon*; yet their hybrid has much the most regular meiosis of any. We may conclude, therefore, that the meiotic abnormalities in the hybrid are not produced by the same causes which produce incompatibility between the species, nor are they the direct result of the evolutionary changes which have separated the species from each other. A fuller understanding of their significance depends, therefore, on an analysis of each of the more important abnormalities in turn.

*Inversions.*—The significance of inversions in producing variability within the species has already been discussed. The frequency with which inversion heterozygotes occur in both animals and plants is amply exemplified by the numerous cases now on record (DARLINGTON 1937, p. 274) even though the method of identification of this type of structural hybridity is a relatively new one; while the studies of STURTEVANT and DOBZHANSKY (1936) on *Drosophila pseudoobscura* give direct evidence of the correlation of inversions with geographic distribution. Hence inversions must be considered one of the important causes of variation within the species. That they are much less important in producing complete isolation and discontinuity in morphological characteristics is certainly true in *Paeonia* although in *Drosophila* they seem to be important in this respect also (DOBZHANSKY and TAN 1936).

*Multivalent configurations.* The most striking feature of all but two of these diploid hybrids is the frequent appearance of multivalents of the type resulting from the pairing of reduplicated segments or from small translocations. Although several points have already been advanced in favor of reduplications rather than translocations as the cause of these multivalents, one point may be considered further here, the correlation between the amount of asynapsis and the frequency of multivalents. This is particularly striking in the triangle of hybrids involving *P. albiflora*, *tenuifolia*, and *anomala Veitchii*. The hybrid *P. tenuifolia* × *Veitchii* is the only one of this triangle in which the chiasma frequency is less than one and the average number of univalents per nucleus is more than two, and also the only one in which multivalents occur with any frequency. These multivalents are of three types:

1. Trivalents or quadrivalents of which all the components have median constrictions; no inversions evident (fig. 9D).
2. Trivalents involving two median and one submedian (D) chromosome, the latter always paired inversely with one of the other two (fig. 9A; 9B).
3. Trivalents involving one subterminal (E) and two median chromosomes; no inversions evident (fig. 9E).

If these multivalents are the result of translocations, then we might say that the genom of *P. anomala Veitchii* differs from that of *P. tenuifolia* by three translocations.

On the same basis *P. anomala Veitchii* differs from *P. albiflora* by only one small translocation. Hence *P. albiflora* should differ from *P. tenuifolia* by at least two translocations. But four different plants of *P. albiflora*  $\times$  *tenuifolia*, in spite of their high chiasma frequency, form no multivalents whatever, indicating that *P. albiflora* differs from *P. tenuifolia* by no translocations at all. Hence the assumption of interchange clearly does not explain the observed phenomena. On the other hand, if we assume that in *P. anomala Veitchii* a segment AB, occurs in both an A, B, or C and a D chromosome pair; that another, CD occurs in two different median pairs; and that a third, EF, occurs in the E as well as in A, B, or C chromosome pair, we may explain the phenomena as follows. In *P. anomala Veitchii* itself AB, CD, and EF pair only with those homologous segments located in chromosomes which are otherwise completely homologous, and hence multivalents never occur. Nevertheless, every gamete of *Veitchii* possesses two segments each of AB, CD, and EF, which therefore have entered into the hybrid with *P. tenuifolia*. In the latter species, however, none of the chromosomes are completely homologous to those of *Veitchii*, and therefore the gamete of *P. tenuifolia* contributes to the hybrid corresponding segments that may be denoted A'B', C'D', and E'F', which have a weakened affinity for AB, CD, and EF, and which are probably not reduplicated. Hence in this hybrid the two AB segments derived from *Veitchii* have a greater affinity for each other than either has for A'B' and they can be expected to pair occasionally, as can also the two CD and EF segments. However, since the majority of the chromosomes in which these segments are located still have a weak affinity for their homologues derived from the opposite parent, pairing between them will occur, and multivalents will therefore be produced. A similar explanation, reduction of competition, was used by CATCHESIDE (1932) to explain bivalent formation in a haploid *Oenothera*. On this basis, multivalent formation due to reduplication should be increased by a lowering of the chiasma frequency, and this is exactly the case in *Paeonia*.

There are apparently differences between the species in the amount of reduplication. *P. Delavayi* does not have a significantly lower chiasma frequency than the other diploid species, yet pairing of reduplicated segments occurs in both the plant studied by DARK (1936) and those in the SAUNDERS garden. Since *P. Delavayi* has been in cultivation for only a short while, and the plants are all derived from seeds obtained by a few expeditions to Western China, there is considerable possibility that these two plants are fairly closely related genetically. Therefore it is likely that

both have a similar set of reduplications, which are more numerous than those in the other species. In this connection may be noted the large number of different reduplications which can be detected in *P. Delarayi* var. *lutea*  $\times$  *P. suffruticosa*, "Argosy." In this hybrid configurations have been drawn representing the following reduplications:

1. The distal ends of the E and of an A, B, or C chromosome, not inverted (fig. 10A).
2. The distal ends of the long arms of an E and of a D chromosome, inverted (fig. 10E).
3. The proximal portion of the long arm of an E and the distal of a D, inverted (figs. 10F, 10H, and probably 10G).
4. The distal ends of the long arms of the D and of an A, B, or C pair of chromosomes, not inverted (fig. 10C, and fig. 11A, rightmost chiasma). That this is actually a reduplication and not a case of segmental interchange is made evident by the occasional association of the two D chromosomes by terminal or nearly terminal chiasmata.
5. The short arm of a D and a proximal segment of an A, B, or C chromosome, not inverted (fig. 10B).
6. The distal, or nearly distal segment of the long arm of a D and an interstitial segment of an A, B, or C chromosome, inverted (fig. 11A, at left, and one unpublished figure).
7. Interstitial segments of two median chromosomes, inverted (figs. 10D and 11B).

There are probably other reduplications whose presence has not been detected. That the multivalent formation involving so many different segments is not due to more or less random non-homologous pairing is made clear by the frequent recurrence of the same type of configuration, in spite of the large number of different ones found.

Multivalent configurations similar to those found in these diploid species and hybrids of *Paeonia* have been found in diploids of *Tradescantia bracteata*, (DARLINGTON 1929) and *Matthiola incana* (PHILP and HUSKINS 1931), while in trisomic forms of *Datura* (BELLING and BLAKESLEE 1924) and in *Drosophila* (BRIDGES 1935) there is evidence for the presence of reduplicated segments within the same chromosome.

They are therefore probably of occasional occurrence throughout the plant and animal kingdoms, but in most organisms can be detected only under very unusual conditions. Two explanations can be given for the unusually abundant evidence for their existence in these hybrids of *Paeonia*.

1. Reduplications are actually more frequent in *Paeonia* than in most plants. This could explain in part the remarkable length of the chromosomes in this genus.



2. Due to the great length of the chromosomes and the relatively small size of the nucleus when zygotene pairing begins, the chromosomes are all closely crowded together, and hence, with reduced competition, homologous segments of non-homologous chromosomes have an unusually good chance of coming together. There is of course no way of deciding which of these is the true cause of the frequency of multivalents, and both may be partly responsible for it. Since BRIDGES (1936) has shown that a "mutation" in *Drosophila* is the result of reduplication, this may be an important factor in the evolution of new forms in *Paeonia*.

An interesting fact is that the species and hybrids with a great amount of structural hybridity do not have a lower proportion of terminalized chiasmata at metaphase than those in which there is less hybridity. This supports the opinion of DARK (1936), that chiasma movement in *Paeonia* is very slight. With an equal amount of movement of chiasmata, one should expect a larger proportion to be terminal in hybrids with a genetically induced reduction in pairing. Since as MCCLINTOCK (1933) has shown pairing begins at the ends of the chromosomes both where the homology is strong and where it is weak, a failure of the chromosomes to complete synapsis would reduce chiasma frequency in their middle parts more than at the ends. This would result in the formation of a relatively high proportion of chiasmata distal to the non-homologous segments, and thus could compensate for the reduction in terminalization due to change of homology. Although there are no good observations of prophase stages to support this hypothesis, partial examination of three nuclei of *P. tenuifolia*  $\times$  *triternata* at early to mid-diplotene showed the presence of terminal chiasmata already at this stage, even though the total number of chiasmata was very small.

#### *Chromosome pairing and interspecific relationships*

Two common criteria for determining the distinctness of species from each other are the amount of sterility in the hybrids between them, and the extent to which their chromosomes fail to pair. In *Paeonia*, these two criteria are useful to only a limited extent, that is, to distinguish between the varietal or subspecific and the specific status of a form. There is a sharp distinction between the complete fertility of such hybrids as *P. Delavayi*  $\times$  *Delavayi* var. *lutea* and *P. anomala* *Veitchii*  $\times$  *Woodwardii* and the almost complete sterility (at least so far as seed setting is concerned) of all of the true interspecific hybrids. This is undoubtedly a valuable asset in the delimitation of species.

Beyond establishing the distinctness of the species from each other, however, the amount of pollen sterility and of asynapsis have little or no correlation with the degree of morphological difference between them. The

*albiflora-tenuifolia-anomala Veitchii* triangle illustrates this point. By far the greatest amount of asynapsis occurs in *P. tenuifolia*  $\times$  *anomala Veitchii*; hence on genetic grounds these two species should be considered the most remote from each other, with *P. albiflora* occupying a position between them. On morphological grounds, however, the three species are more or less equidistant, but *P. anomala* is somewhat intermediate and nearer to *P. albiflora*, while *P. tenuifolia* is particularly remote from the latter species with which it forms a hybrid with almost perfect pairing. The difference between these species are discussed elsewhere (SAUNDERS and STEBBINS 1938; STEBBINS 1938). There is a somewhat closer correlation in the case of *P. triternata Mlokoewitschii*, which is quite remote taxonomically from both *P. anomala* and *P. tenuifolia*, and forms with both of them hybrids having a very irregular meiosis.

Nevertheless, the pairing affinity of the chromosomes is of little or no value for determining phylogenetic relationships between the species of *Paeonia*. Equally useless in this respect are both the morphology of the somatic chromosomes and the degree of structural differentiation between them as determined by the "structural hybridity" of their hybrids. Although there is some correlation between structural hybridity and failure of pairing, the former is probably of minor importance as a cause of the latter since in many species and hybrids one or the other is present by itself.

For example, the heterozygosity for many small inversions of *P. Delavayi*, *anomala*, and *triternata* does not reduce the metaphase chiasma frequency of these species at all; in *P. albiflora*  $\times$  *anomala Veitchii* a smaller amount of structural hybridity is associated with a great reduction in chiasma frequency; while *P. albiflora*  $\times$  *tenuifolia*, in which there is a considerable reduction in chiasma frequency, a marked reduction in pollen fertility, and practically no seed setting, shows the presence of only one inversion.

Asynapsis, therefore, is probably due to genic differences, but these differences are more or less independent of the ones responsible for the observable morphological differences between the species, and have been important chiefly in producing genetic isolation, with the consequent independent mutation of different ancestral stocks separated by such genic factors for sterility and for failure of chromosome pairing. Further evidence for this interpretation of the significance of asynapsis in the hybrids is that those hybrids with the least irregular meiosis are between species very well isolated from each other in other respects, and vice versa. For instance, *P. tenuifolia* and *P. albiflora* have in nature always been separated from each other geographically by thousands of miles; their periods of blooming are a month apart; and they are very difficult species to cross

artificially (SAUNDERS and STEBBINS 1938); on the other hand *P. tenuifolia* and *P. triternata* occur in the same region, bloom at the same time, and are relatively easy to cross; but are isolated by the extremely abnormal meiosis and the complete sterility of the hybrid between them. In *Aquilegia* (ANDERSON 1931) specific isolation is produced by geographic barriers alone; in *Tradescantia* (ANDERSON and SAX, 1936) it is produced in part by sterility factors independent of chromosome pairing, while in *Paeonia* it may be produced either by geographic barriers, by different periods of flowering, by incompatibility in mating, by cytogenetic incompatibility which results in asynapsis and pollen sterility, or to a less extent by gross structural differences in the chromosomes. In *Paeonia*, therefore, as in *Aquilegia*, and probably most other genera, the evolution of the diploid species has been brought about primarily by the accumulation of a large number of small genetic changes, which for lack of a better understanding of them may be termed gene mutations. These are responsible for changes in external morphology and in the physiological makeup of the species. In addition, genetic changes affecting chromosome behavior at meiosis as well as the development of the gametophyte have occurred, but these have been independent, at least in the frequency of their occurrence, of the mutations responsible for morphological changes. In many cases, however, they have produced the isolation necessary for the evolution of two or more different species in the same region. The third type of change—gross alteration of the chromosome structure by means of inversions, reduplications, and translocations—has occurred very frequently, inversions having been much the most frequent. Alterations of this type have been responsible chiefly for the “group-discontinuity” which has formed the various forms or varieties of *P. Delavayi*, *P. anomala*, and *P. triternata* but, unaccompanied by other agents, have not been effective in the actual differentiation of species.

The writer wishes to express his thanks to Dr. A. P. SAUNDERS for supplying the material for this study, and for much technical assistance.

#### SUMMARY

1. The somatic chromosome number of the species of *Paeonia* is either  $2n = 10$  or  $2n = 20$ , and the chromosomes are similar in size and morphology in all of the species.
2. Certain abnormalities of meiosis, chiefly the occurrence of fragments and chromatin bridges as a result of crossing over in inverted segments, are found in all of the species, but are most abundant in *P. triternata*, *anomala*, and *Delavayi*, and less so in *P. albiflora*, *Emodi*, and *suffruticosa*.
3. The metaphase chiasma frequency ranges from 1.58–1.93 in the various species, and the terminalization coefficient from 0.41–0.52. There

is no correlation between the relative chiasma frequencies at metaphase and the percentage of asynapsis.

4. In one form of *P. anomala* multivalents resulting from segmental interchange involving a large segment of a chromosome were found.

5. Of the diploid hybrids, *P. albiflora* × *tenuifolia* and *P. albiflora* × *anomala Veitchii* show few abnormalities of meiosis except a lower chiasma frequency and a higher percentage of univalents, but in the others a relatively high frequency of bridge-fragment configurations and of multivalent configurations containing triple or quadruple chiasmata occurs. This indicates that these hybrids are heterozygous for a large number of small inversions and for duplications of segments. These are analyzed in one hybrid, *P. Delavayi* var. *lutea* × *suffruticosa*, and several different types of configurations resulting from the presence of inversions and duplications in the same chromosome group are described.

6. The three species which are heterozygous for a relatively large number of inversions are morphologically relatively polymorphic, while the others, except for the existence of horticultural varieties, are less variable morphologically. This is in agreement with the postulate of DARLINGTON on the effect of small inversions on morphological variability.

7. The apparently higher chiasma frequency of *Paeonia* species and hybrids grown in central New York as compared with the same forms grown in England is ascribed to climatic differences between the two regions.

8. The relatively large number of duplications found in *Paeonia* species is made particularly evident in hybrids with a low chiasma frequency, due to reduction in competition. Evidence against the interpretation of the multivalents found as resulting from interchange of many small segments is presented.

9. The degree of pairing and the amount of sterility in the various *Paeonia* hybrids is little or not at all correlated with the degree of morphological similarity between the parent species. Asynapsis and sterility have been important factors in producing isolation between the species, but have taken place more or less independently of the mutations responsible for the morphological differences between them.

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# SALIVARY ANALYSIS OF INVERSION-3R-PAYNE IN THE "VENATION" STOCK OF *DROSOPHILA MELANOGASTER*

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Received September 13, 1937

## INTRODUCTION

THE recessive mutation "venation" was found by C. B. BRIDGES and shown by genetic tests to be associated with a dominant suppressor of crossing over, both lying in the right limb of chromosome III. Salivary analysis by P. N. BRIDGES in the summer of 1936 showed that this suppressor is a simple inversion, with break points in 89C and 96A. Shortly after this, C. B. BRIDGES and J.-C. LI studied inversion 3R-Payne and found similar break points. This paper reports the accurate determination of the salivary characteristics of the Payne-R inversion. Of all the inversions known for *Drosophila melanogaster*, Payne-R is the one most frequently encountered in wild stocks collected at widely separated localities (STURTEVANT 1931) and is now the one most widely disseminated through the stocks of mutations, where it is a very useful balancer of third chromosome mutants or constitutes a troublesome impurity in at least ten percent of the stocks of first, second and fourth chromosome mutants. The Payne inversions have been very extensively studied genetically, and now, with this accurate determination of the salivary limits of Payne-R, should increase in significance and usefulness.

## THE MUTATION "VENATION" AND ITS ASSOCIATED REDUCER OF CROSSING OVER

In the balanced stock of *fu/CIB* some of the fused and heterozygous Bar flies showed (July 18, 1933) a spontaneous mutant character. This was called "venation" since the most easily seen characteristics were an irregular thickening and a slight diffuse branching of the veins, especially of L<sub>3</sub> and of the crossveins which were closer together. There were several other ill-defined characteristics: bulging, scarlet-toned eyes; gnarled bristles; small size, and, as breeding tests soon showed, frequent sterility and a viability only about half that of normal (BRIDGES 1937).

F<sub>2</sub> cultures from the cross of venation to Plum Stubble gave a random distribution of venation with Plum (chromosome II) and gave 431 *Sb* to 104 *ven* flies, showing that venation is a low-viability recessive in the right limb of III. Testcross cultures of venation against Dichaete<sup>3</sup> Hairless

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gave:  $D^3 H = 373$ ,  $ven - 201$ ,  $D^3 ven = 7$ ,  $H = 19$ , agreeing with the hypothesis of a low-viability recessive in IIIR, but showing that it is associated with a dominant inhibitor of nearly all the crossingover in that limb. A stock of venation balanced over Delta<sup>5</sup> was established (Stock 738, Drosophila Information Service 7:33).

#### LETHAL WITH VENATION

Some of the  $F_2$  cultures of the cross of  $ven \times Pm Sb$  gave the following totals:  $Sb = 347$ ,  $ven = 3$ , indicating that the parental venation fly had been heterozygous for a recessive lethal (reported, BRIDGES 1937, as  $l(3)36d24$ ; kept in balanced stock 739, Drosophila Information Service 7:33). That the lethal is separable from venation and occupies a locus near the spindle attachment of III was shown by crosses of  $D^3 H/l ven \text{ } \varnothing \times l ven/Sb \text{ } \sigma$ , which gave (after discarding all  $Sb$  offspring): 557  $D^3 H$ , 27  $H$ , 3  $D^3 ven$ , and 3  $ven$ . This position was confirmed by the cross  $D^3 l ven/H \text{ } \varnothing \times l ven/Sb \text{ } \sigma$ , which gave the following non- $Sb$  offspring: 195  $H$  and 27  $D^3 H$ .

#### SALIVARIES OF VENATION

Permanent aceto-carmin preparations from female larvae of the type  $ven/D^3 H$  showed a medium-sized inversion loop in 3R (fig. 1a). The inversion includes nearly a third of IIIR, with the first break-point in 89C, just short of the middle of IIIR, and the second break in 96A, about one-fifth down from tip of IIIR. Highly characteristic of this inversion is the inclusion of the easily-recognized "duck's head" region, with the "weak spot" (at the tip of the "bill") very close to the right of the break.

Preliminary to a precise determination of the break points it was advisable to restudy and remap the normal banding in the neighborhood of the breaks. In figures 1b and 1c are given revisions of sections 89 and 90 and of 96, drawn from exceptionally clear well-stretched large chromosomes.

Study was centered on those heterozygous inversion figures in which a flat "open-square" configuration is formed at the junction point of the loop with the rest of the chromosome (figs. 1d and 1e). In such cases a direct comparison can be made between the two normal regions and the two inversion sequences. Furthermore, the synopsis of homologous bands helps to check the point at which change of partners occurs. It was found that the first break-point follows directly after the heavy doublet which begins 89C and precedes the three weak doublets of that subsection. The second break is about three-quarters of the distance along 96A, between two fairly strong doublets.

In a heterozygous inversion the crowding and bending of the strands at the junction point tend to obscure the banding, and also it is rare that the



FIGURE 1.—Salivary chromosomes of Inversion-IIIIR-Payne. a. General topography of IIIIR of heterozygote. b-c. Revision of normal map for sections 89, 90, and 96, showing left and right break-points. d-e. Details of 4 strands at open-square junction in heterozygote (e enlargement of a). f-g. Homozygote, left abutment. h-i. Homozygote, right abutment.



loop itself is stretched enough to show the banding clearly. In a homozygous inversion there is no loop and the regions at the break-points can be examined in fully stretched undistorted condition. In preparations of homozygous venation with its inversion the easiest point of recognition of the inversion is the displacement of the "weak point" and "duck's head" from their normal central position in IIIR to a point four-fifths toward the free end.

Study of the region of the left break-point in the homozygous inversion (figs. 1f and 1g) showed the bands of 89 running through the strong doublet of 89C. But this doublet is now closely followed by a fairly strong doublet instead of by the normal sequence of three faint doublets. Then follows the characteristic banding of 96A and 95, in reversed sequence. The reversed sequence continues through 90 and through the faint doublets of 89C where, at the second break-point (figs. 1h and 1i), it abruptly encounters the fairly strong second doublet of 96A. The bands then proceed in normal sequence through the rest of 96 and on to the tip of IIIIR.

A comparison of the break-points of the IIIIR-inversion of the venation stock with the break-points of the highly important In(IIIIR) Payne, which had been worked out somewhat later by C. B. BRIDGES and J.-C. LI, showed the two inversions to be identical. However, the In(IIIIR) $P^{ven}$  is free from a lethal which is inseparable from In(IIIIR)P in the balancers "Payne" and "Payne, *Dfd ca.*"

#### SUMMARY

The recessive mutant "venation" (*ven*) has its locus in chromosome IIIIR and is associated with a simple inversion. Salivary analysis of this inversion in heterozygous and homozygous form shows that it is the Payne-R inversion. Careful study places the left break-point of this important inversion directly after the heavy doublet which begins 89C, and the right break-point between the two fairly strong doublets at about three-quarters of the distance along 96A.

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# GENETICAL STUDIES ON THE WAX MOTH *GALLERIA MELLONELLA* LINN.<sup>1</sup>

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Received September 15, 1937

## INTRODUCTION

THE wax moth, *Galleria mellonella*, is found wherever bees are found. Some phases of its general biology have been worked out by METALNIKOV (1908), PADDOCK (1918), BORCHERT (1933) and VOHRINGER (1934), but it has not been used previously for studies in genetics. Its viability even under adverse conditions, its highly prolific nature, and its general adaptability to the laboratory account for its being chosen for this investigation.

More than thirty mutants have already been obtained following X-ray treatments; many mosaics for mutant traits have been found and several gynandromorphs; and definite evidence for facultative parthenogenesis in about ten percent of isolated virgin females has been obtained. These results indicate the usefulness of this new material for several unsolved problems in genetics.

## HISTORY AND TAXONOMY

The wax moth has been known since very early times. ARISTOTLE gave a fair description of it in his *Historia Animalium*, Book VIII, Chapter 27 (SMITH and ROSS 1910). Occasional reference is made to it throughout the Christian era. According to PADDOCK (1918) it was probably introduced into the United States from Europe about the middle of the nineteenth century. It is universally known to the apiarist as a pest.

This moth is the only species in its genus. It is also physiologically isolated, since in its food taking (larval) stage it eats only old bee combs. The taxonomic designation used here is that indicated by PACKARD (1898), COMSTOCK (1924), and IMMS (1924).

## LIFE HISTORY

*Galleria* will breed and produce offspring the year round over a very wide range of environmental temperatures. The optimum temperature is around 30 to 35 degrees centigrade. At this temperature a generation cycle is completed in about eight weeks, thus one is able to obtain six or seven generations a year. The duration of the life cycle is roughly inversely proportional to the environmental temperature. The eggs, which range in

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number from 200 to 1,000 per female, require about ten days to hatch. The larval stage, which has eight instars, is about four weeks in duration; the pupal stage about twelve days.

#### CULTURE METHODS

The moths are cultured in half-pint milk bottles kept in an aerated temperature-controlled incubator. An open vessel of water prevents excessive drying of the air. Instead of the cotton plug for a stopper the regular waxed cardboard milk bottle caps are used. These fit snugly and prevent the small larvae from escaping. The moths used for mating are placed by pairs in clean bottles with about two cubic inches of old brood bee combs. Since the moths mate very soon after eclosing, it was found best to isolate the females while still in the pupa case to insure their virginity. The larvae are ravenous eaters throughout their larval life. As food is consumed, additional pieces of combs are added. When the larvae reach about a centimeter in length they are subcultured. The larvae of a given pair mating, numbering 200 or more, are distributed into several bottles of about fifty larvae each. From this stage to eclosion, each bottle is stoppered with a special ventilator cap. These consist of two regular caps through the center of which is punched a one-inch hole. A circular piece of 40 mesh copper wire screening, with the diameter of the regular caps, is placed between the punched caps, which are then stitched together with a stapler. This metal screen permits access of fresh air into the bottle and also prevents the larvae from nibbling their way out. A more detailed account of the culturing methods and a peculiar ability of larval colonies to raise their own common temperature many degrees above that of their environment will be described in another paper.

In handling the eclosing imagoes anesthetization is unnecessary. After eclosing, the individuals sit motionless as long as it is daylight. Thus it is easy to scoop them out one by one by means of the special "moth dipper." The "dipper" is made of a short shell vial,  $\frac{3}{4} \times \frac{3}{4}$  inches, glued to the end of a thin strip of wood about the dimensions of a tongue depressor. If the dipper is gently placed over the moth and moved slowly so that the moth's head strikes the edge of the inside of the dipper, the animal will generally shift its feet, re-anchor them on the side of the dipper and then remain as quiet as before. Sometimes this gentle disturbance will cause them to make one or two circles and then come to rest either on the side walls or the bottom of the dipper. One may then withdraw the dipper from the bottle and carry it with the moth to any part of the room or observe it in any position under the binocular or hand lens without disturbing it. For individual handling and observation it has been found convenient to transfer each

moth to an inverted cardboard milk bottle cap. The moths so left will generally remain there, unless disturbed, until night fall, at which time they may fly away if not covered with a vial or placed in a culture bottle.

#### PRODUCTION OF MUTATIONS

Since it has been demonstrated that X-rays provide an effective means of inducing mutations (MULLER 1928, STADLER 1928, TIMOFEEF-RESOVSKY 1929 and others), it was decided to try this type of treatment on *Galleria*. The moths, the original stock of which had been obtained from Professor I. R. TAYLOR of Brown University, were inbred (brother-sister matings) for two or three generations in order to detect any recessives that might be contained in the wild stocks. No abnormal individuals appeared.

Two different X-ray machines were used, one at the Crocker Cancer Research Laboratory, Columbia University, the other at the Carnegie Institution, Department of Genetics, at Cold Spring Harbor, New York.

Since the X-ray treatments were used merely as a means to obtain mutants, no detailed statement of the irradiation methods will be given. In various experiments, the strength of dosage ranged from 250 to 5,000 r units. Table 1 gives the dosage which had been used in each case of mutation. This ranged from 1,200 to 4,500 r units. It should be understood that, while all the mutants found were in individuals which had at least one treated ancestor, it is not certain that all the mutations were brought about by irradiation. Offspring of treated individuals were closely inbred and were frequently given additional treatments. After the initial stages of the work, most of the moths observed came from treated ancestry. The treatment of larvae, which was first tried, was later given up. At the suggestion of Dr. H. J. MULLER imagoes were treated with more satisfactory results.

From one series of treated larvae, two mutant characters appeared in the  $F_2$  generation and bred as recessives. These were red scales (*r*) and a dark colored spot on top of the thorax (*ms*).

#### *Abnormal wing carriage (g)*

Culture M13/2b, the male grandparent of which had received 1,800 r units, produced 19 moths—9 males and 10 females. One male and two females had an abnormality in wing carriage. The mesothoracic or anterior pair of wings, which normally point straight backward during rest, were spread at an angle of about 30 degrees to the median plane of the body and were inclined downward, while the metathoracic or posterior wings, which normally are just under the anterior wings, were raised almost vertically medial to the anterior wings. This character is thus very easily detected. The male was mated to a normal sister and the females to normal brothers.

The progeny were all normal. Three brother-sister matings of these  $F_1$  progeny produced a total of 131 moths among which were 8 males and 20 females which showed the wing abnormality.

A male with the abnormal wings mated to a heterozygous sister produced 85 moths of which 14 were *gg* males and 23 *gg* females. A male and a female, both of which had the abnormal wing character, mated with each other produced 46 moths all of which had the wing abnormality. It was concluded that this characteristic was a recessive mutant. Reciprocal matings were made with homozygous wild stocks and no indication was found of sex linkage.

#### *Light (l)*

Culture  $M_{13}/2h$ , a mass culture, the male grandparent of which had received 1,800 r, produced one male, out of a total of 30 offspring, which had a very light color all over the body. This male mated to two females from  $M_{13}/2h$  produced all normal progeny. Three brother-sister matings of these produced 23 males and 17 females with light bodies out of a total of 197 progeny.

#### *Rolled (rl)*

Culture  $M_{13}/2g$ , a sister culture to the above, produced 30 males and 27 females. One male had peculiar-shaped scales, especially on his wings. His body color was the "light" mentioned above. The scales instead of being normally flat were rolled upward around their long axis. This abnormality was termed "rolled" and given the symbol "*rl*." This male was mated to his sister and all the  $F_1$  offspring were normal. The  $F_2$  contained 37 rolled (18 males and 19 females) among a total of 181 progeny.

Rolled appeared simultaneously in a male of another culture,  $M_{10}/2c$ , whose male grandparent had been treated with an X-ray dosage of 2,000 r units. From the offspring of this moth with his sister a brother-sister mating produced 42 moths of which 6 males and 4 females had rolled scales. It was assumed that the parents of this culture were each heterozygous for rolled. From this culture two reciprocal backcrosses among the phenotypically normal brothers and sisters produced 19 rolled males and 19 rolled females out of a total of 92 progeny. From these and other subsequent data the rolled scale character was regarded as an autosomal recessive.

#### *Light tegulate (lt)*

In Culture  $Org/3M_1$  occurred three abnormal females which were  $F_2$  offspring from a female which has been treated with a dosage of 2,200 r units. These females had abnormally light-colored tegules. They were mated to their normal brothers. One of the cultures was sterile. The other

two produced a total of 131 progeny among which were 19 males and 27 females with light tegules.

*Mesoscutellar spot (ms)*

One character which has appeared in several of the stocks may have been present in the stocks or may have come from some of the first larvae that were treated. It is a patch of very dark colored scales covering the mesoscutellum. A male and a female with this character, descendants of the above treated larvae, were mated to a normal female and male respectively. These cultures produced 48 males and 33 females, all normal. One brother-sister mating was made from each of these cultures and produced 133 moths of which 14 males and 19 females had the mesoscutellar spot.

*Pink mesoscutum (me)*

Among the  $F_2$  offspring of culture FO3d/1a, the female of which had been treated with 1,500 r units, occurred one female which had very light pink scales on the mesoscutum. This character is very obvious since the rest of the scales are dark bronze. This female mated to her brother produced 46 males and 27 females which were normal and 30 males and 32 females which had the pink mesoscutum. From this culture an "me" male crossed with his "me" sister produced 17 males and 19 females, all "me."

*Red body (r)*

A female from FO1g/1a, whose mother was treated with 2,250 r units, produced four offspring, one male and three females. One female was a deep, rich red all over including the abdomen. She was mated to a normal male, which was also an offspring from the above treated larvae, and produced all normal progeny. The  $F_2$  consisted of 32 normals and 11 reds (5 males and 6 females).

*Non-red body (nr)*

In an  $F_2$  generation of 49 non-red scales (nr) from Culture M31/1b the male of which had received 1,200 r units there occurred four males and two females all of which lacked any trace of the bronze or scattered reddish scales which are typical of the wild type. These, when isolated and mated, produced 92 males and 47 females; all progeny were non-red. From subsequent stocks a red male mated to a non-red female produced normal wild males and females. A brother-sister mating of these offspring (heterozygous for both traits) produced only three types: normals, 10 males and 11 females; reds, 2 males and 3 females; non-reds, 7 males and 2 females; or 21 normals, 9 non-red, and 5 red. While this may be a 9:3:4 type of dihybrid  $F_2$ , further analysis is needed.

*Eosin eye (e)*

In Culture M<sub>13</sub>/3b<sub>5</sub>, the male great grandparent of which had received 1,800 r units, a male appeared which had eosin colored eyes. He was mated to three different normal females and produced 179 males and 160 females all wild type. In the F<sub>2</sub> there were 261 normals (127 males and 134 females) and 89 eosin (52 males and 37 females). This character was classed as an autosomal recessive.

*Vermilion eye (v)*

A vermilion-eyed male appeared in culture 13/5b<sub>5</sub>43. It had come from two lines, the males of which two generations back had received 2,200 r units and 2,250 r units respectively. This male mated to wild type females gave F<sub>1</sub>'s with wild eye color. Six F<sub>2</sub> cultures produced a total of 938 moths among which were 89 males and 89 females with vermilion eyes. This result indicates that vermilion is inherited as an autosomal recessive.

*Maroon eye (m)*

A dark reddish color of the eye, which was called "maroon" first appeared in a female in culture M<sub>10</sub>/6c<sub>25</sub>11. Her paternal ancestor two generations back had been treated with 2,000 r units. This female was crossed with a normal brother and the F<sub>1</sub> was normal. The F<sub>2</sub> consisted of 216 normals and 85 maroons. This ratio suggests that maroon is inherited as an autosomal recessive.

*Shovel (s)*

In culture M<sub>13</sub>/4b<sub>40</sub> a male was found in which the wing scales were shovel-shaped and had only one central spine at the distal border. The normal scale is slightly oblong with five distal spines, a slightly longer median one with two progressively shorter ones on each side of it. The F<sub>1</sub>'s from this male were normal. One pair of these were mated, but the culture was sterile. This same character appeared also in culture TM<sub>23</sub>/21, which was an F<sub>2</sub> mating from a male which had received 4,500 r units. In another F<sub>2</sub> culture (TM<sub>23</sub>/28) from the same male there were 4 males and 2 females (shovel) among a total of 49 offspring. Of the offspring of the male from TM<sub>23</sub>/21 three brother-sister matings were made. Two of these cultures produced 175 moths of which 42 had shovel scales.

From culture TM<sub>23</sub>/28 the four males were mated to their normal sisters. Three of these crosses produced only normals; the fourth produced 37 shovels out of a total of 81 imagoes. The two shovel females, which eclosed after the four males were mated, were mated to their normal brothers. These two cultures produced 63 shovel-scaled individuals out of a total of 136 offspring. The treated males in this case came from culture

M<sub>13</sub>/4b40, which was a culture from previously treated moths, so that the shovel character may have arisen from the earlier treatment.

#### ABNORMALITIES FROM TM SERIES

It was determined by a series of test treatments ranging from 3,000 to 5,000 r units that a sub-sterilizing dose for males was around 4,500 r units. Dosages of 3,500 to 4,500 r units were given to 33 males. This group is called the TM series, each of which was mated to a virgin female. Of these, three cultures were sterile. The remaining 30 cultures produced from 3 to 77 offspring each. Among the total of 1,319 F<sub>1</sub> progeny from these crosses, there were three abnormal males and eleven abnormal females.

##### *Black body (bl)*

Of the three abnormal males, one had a black or ebony-colored body. When mated to a sister he produced 29 normal moths and 2 black females. These two females were mated to their normal brothers and produced only normal progeny. Two brother-sister matings of these progeny produce eight black-bodied moths among a total of 76 progeny.

In the other two males with abnormalities, the characters did not reappear in later generations.

##### *Needle-scales (nd)*

Of the eleven abnormal females, two proved to be recessive mutants, namely needle-like scales, and chianti-colored eyes. The needle scales, which are long and narrow with one to three short terminal spines, were found in a female of culture TM-34, the male parent of which had received 4,000 r units. She was mated to a normal brother and produced normal offspring. Two cultures of these offspring produced 27 needle-scaled moths among a total of 168 offspring.

##### *Chianti eye (ch)*

The chianti-colored eye occurred in a female in culture TM28, the male parent of which had received 4,000 r units. Mated to a normal brother, she produced two chianti females in a small culture of 23 offspring. These two females mated to normal brothers produced normal offspring. Three brother-sister matings of these offspring produced 41 chianti-eyed moths among a total of 270 offspring.

#### EYE COLOR MUTANTS

In the F<sub>2</sub> and F<sub>3</sub> generations of the above series of treated males, there have arisen several abnormalities among which are a series of eye colors ranging from a normal black eye tinged with red through several distinct steps of increasing degrees of lighter red to pure white. In addition to this



series there have occurred a colorless eye, which has the appearance of clear glass or cellophane; a bright yellow eye; a gold eye, which gives the appearance of highly polished new gold; and one which has the appearance of corroded old gold.

In order to designate the various eye colors a standard color dictionary (MAERZ and PAUL, 1930), was used. The color name in each case is that used by the dictionary and the reference for color-chart identification is given in parentheses after each color name and symbol. The eye colors found in this series include the following: canyon, *ca*, (7E6); chianti, *ch*, (6L6); salvia, *sa*, (3L6); rose of sharon, *rs*, (3K8); jasper pink, *ja*, (2I8); roseleaf, *rf*, (2G8); reveree, *re*, (2B8); white, *w*, (3A1); yellow, *y*, (10I4); sorolla brown, *sb*, (6A12); spanish cedar, *sc*, (6J10); glassy, *gl*; old gold, *go*; and new gold, *gn*.

#### *White eye (w)*

The white eye occurred in three different cultures: the first in the  $F_2$  from culture TM17, the second in the  $F_3$  from culture TM23, and the third in the  $F_3$  from culture TM9. In each case the male parent had received a dosage of 4,000 r units. The three cultures in which white eyes occurred produced a total of 196 offspring among which were 21 white (11 males and 10 females). The successive male progenitors of the above cultures had been treated with the heavier dosages of X-rays. The data indicated that at least two of the three whites had arisen independently.

#### *Canyon eye (ca)*

The canyon-colored eye appeared in a female of culture TM28/22, the male grandparent of which received 4,000 r units. The culture was an  $F_2$  and produced 9 males and 8 females, one of which was canyon. The treated grandparent in this case was a direct descendant of culture M13/2b, in which had appeared, six generations earlier, the maroon-eyed male mentioned previously. At that time no color standard was at hand. It is thought that "maroon" and "canyon" might be the same. There was no chance to breed the two together since the maroon stock had been lost. This eye color gene may have been carried in the strain from the previous irradiation. The canyon-eyed female mated to her brother produced normal offspring. Two  $F_2$  cultures produced 43 canyon-eyed moths among a total of 226 progeny.

#### *Salvia eye (sa)*

The "salvia" eye appeared in three males of culture TM28/31 among a total of 45 imagines. These three males were mated to normal virgin sisters. Two of these matings produced all normal offspring. The third produced

19 salvia-eyed moths in a total of 40 offspring. The third sister was apparently heterozygous. This abnormality came from the stock which six generations back had the same ancestor as the stock producing the vermilion eye mentioned above in culture M13/5b543. The vermilion of M13/5b543 appeared to be the same as the salvia in this TM28/31. A cross of two males and a female of the vermilion stock with the salvia stock produced all salvia. The vermilion and salvia were thus regarded as identical or as alleles. It is not known whether the abnormality of culture TM28/31 arose from the treatment given the male of TM28 or had lingered in the stocks from the previous treatment.

*Rose-of-sharon eye (rs)*

The "rose-of-sharon" eye color appeared in culture TM9/11, the male grandparent of which had received 4,000 r units. It is a degree lighter than salvia and arose in a sister stock to TM9/121 which contained the third observed white eye. It, therefore, has the same treated ancestor on the father's side, and on the mother's side the treated male of TM26 in which the X-ray dosage was also 4,000 r units. It is not known in which ancestral male the mutation occurred. There were two males with this eye color in a total of 24 imagoes. These two males were mated to their sisters. One of these cultures produced 70 normals. The other produced 41 with rose-of-sharon eyes and 62 with normal eyes. Inbred strains of this eye color produced only rose-of-sharon eyes.

*Jasper pink eye (ja)*

A "jasper pink" eye color appeared in two males out of 21 imagoes in culture TM34/31 which was the F<sub>3</sub> from a male of the TM series which had been treated with 4,000 r units. The first male to eclose was mated to a virgin female from TM34/34. Their progeny were all normal. The second male was mated to his virgin sister and produced 17 jasper pink-eyed individuals, 10 male and 7 female, out of a total of 42 offspring. When inbred this character bred true. Reciprocal crosses with wild produced only wild in F<sub>1</sub>. Two F<sub>2</sub> cultures produced 86 normal and 17 jasper pinks.

*Roseleaf eye (rf)*

In culture TM7/11, an F<sub>3</sub> from a male of the TM series which had received 4,500 r units, there was found a male with soft pink eyes. This color was "roseleaf" (2G8) of the color standard. The male ancestor five generations back on the mother's side had been treated with 2,000 r units. This male mated to his virgin sister produced 36 normals. Two brother-sister matings of these produced 49 roseleaf among a total of 189 progeny.

*Reveree eye (re)*

Culture TM<sub>30/11</sub>, the male of which two generations previously had been treated with 4,000 r units, produced five males with whitish eyes, having a pinkish tinge of about the same intensity as the yellowish tinge characteristic of ivory. This, according to the color standard, is "reveree" (2B8). Each of these five males was mated to a normal virgin sister. In three of the cultures all the offspring were normal. The other two cultures produced 35 moths with reveree eyes among a total of 181 offspring.

*Glassy eye (gl)*

From culture TM<sub>7/42</sub> (the original treated male ancestor of which is the same male that gave rise to the roseleaf eye) there appeared one male, out of a total of six individuals, which had a colorless eye that looked watery or glassy. As stated above, this culture on the mother's side had a male ancestor five generations back which had been treated with 2,000 r units. No other strain is concerned in this case since brother-sister matings were used in both subsequent generations from the treated male of culture TM<sub>7</sub>. This abnormality seems to be a condition where the cells in the ommatidia are entirely without pigment. After these moths are left in the light for a time the glassy appearance of the eye changes to the refractive appearance of sand-scratched glass. The interpretation of this is that the ommatidial cells, which apparently are normal except for the pigment, recede in the normal way down the ommatidia when exposed to light, leaving the lens-like facets to refract the light. This glassy-eyed male was mated to two normal sisters. One of these cultures produced 12 males and 9 females with glassy eye and 15 males and 16 females with normal eyes. Two brother-sister matings of normal eyed moths of these produced 35 glassy among a total of 193 offspring. (The glassy-eyed moths bred true.)

*Silvered eye (si)*

Two males in culture TM<sub>17/43</sub> had "silvered" eyes. This is similar to white except that it has a metallic glitter which gives the appearance of highly polished silver. The two silvered males have a pedigree in which many ancestors were irradiated. They were mated to two sisters and produced a total of 151 moths of which 74 were silvered.

*Spanish cedar eye (sc)*

In culture TM<sub>23/2</sub> there appeared three different abnormalities which consisted of one rust and two different gold eye colors. A yellowish rust color, identified in the color dictionary as "Spanish cedar," was found in two females. One of these females mated to a normal male produced 63

normal moths. Three brother-sister matings of these produced 40 Spanish cedar-eyed moths and 174 normals.

*Gold eyes (go) and (gn)*

One of the two golds mentioned above had the glittering, "new gold" appearance similar to silvered. The other appeared as old gold without the luster. Crossing the two different golds gives a mixture of the two eye colors in the  $F_1$ 's, but additional crosses must be made to clear up the situation. In the "new gold" eyes the pigment does not recede from the facets, or at least the shiny appearance never disappears, regardless of how long the moths are left in the light. In the "old gold" eye the pigment seems to recede in the normal way when the moth is in the light. This culture, TM<sub>23/2</sub>, had two males and three females with "new gold" eyes, and one male with non-shiny or "old gold" eyes. The two types were isolated as stocks and in repeated tests have bred as normal recessives.

*Yellow eye (y)*

Culture TM<sub>29/12</sub>, which produced only four males and six females, had one male with lemon-colored yellow eyes. The color corresponds to the 10L4 yellow of the color dictionary. The ancestors of this male include a larva treated with 700 r units; two generations later a male moth treated with 1,800 r units; and three generations later a male treated with 4,000 r units in whose  $F_3$  progeny the yellow-eyed male occurred. This male mated to a sister gave 33 males and 40 females all normal. Two brother-sister matings from these produced 61 normal and 45 yellow.

*Lobed eye (lo)*

A lobed-eye male and female occurred among 31 offspring in culture 13/6b3812. The eyes were normal except for a horizontal depression across the lower half of the eye. The parents in this case were a brother and sister. The mutant male died. The female mated to a normal brother produced only 6 males and 3 females, of which 1 male and 3 females had lobed eyes. The male died within 24 hours. The females mated to normal brothers produced only a few larvae each, from which the character did not again appear. From these very meager data, this character is interpreted to be a recessive and probably semilethal.

*Sorolla brown (sb)*

Culture TM<sub>20/2</sub>, which was an  $F_2$  from a treated male in the TM series, produced two brownish-eyed females in a small culture. This eye color, according to the color standard, is "sorolla brown." The male ancestor five generations back had received 1,800 r units and two generations back,

4,000 r units. The two abnormal females were mated to normal brothers. These two cultures produced 120 moths among which were 36 with "sorolla brown" eyes.

In these TM series there appeared again the ebony body color, the needle scale, and the shovel scale characters. Since the TM series of treated males were descendants of the stocks in which these same characters arose previously, it is not known whether they were new mutations or had persisted in the stocks.

#### LINKAGE

As mentioned above, rolled scales and light body color arose from a common treated ancestor. In isolating rolled it was noticed that such individuals were also lighter colored than normal. It was at first thought that the lighter color of rolled might be an effect of the rolled gene. Such was not the case. Six cultures, in which heterozygotes were backcrossed to light colored rolled individuals, showed the dihybrid nature of the cross with close linkage involved. In these crosses the genetic constitutions were:  $lrl/++$  by  $lrl/lrl$ . In the first four cultures the males were heterozygous and in the last two the females were heterozygous. These six crosses produced the following:

| CULTURE NO. | NORMAL | $lrl$ | $l$ | $rl$ |
|-------------|--------|-------|-----|------|
| 1           | 44     | 36    | 1   | 1    |
| 2           | 37     | 39    | 2   | 1    |
| 3           | 39     | 35    | 0   | 0    |
| 4           | 36     | 31    | 1   | 1    |
| 5           | 43     | 38    | 0   | 0    |
| 6           | 31     | 26    | 0   | 0    |

The rolled-scale normal-colored and the normal-scale light-colored both bred true when inbred.

It will be noted that crossing over occurred in three of the four cultures where the male was the heterozygous parent. While none of the mutant genes in *Galleria* have proved to be sex-linked, it is very probable that in *Galleria*, as in other lepidopteran species, the male is the homogametic sex. The amount of crossing over between rolled and light based on the first four cultures is 2.3 percent. The absence of crossovers among the progeny from the two heterozygous females suggests that crossing over may be less in the female than in the male or may not occur at all. More extensive data are needed to determine whether a real difference exists between the sexes in this respect.

The cross involving rolled and light is the only one in which evidence of linkage has been found. In crosses involving the various combinations of the genes *w*, *s*, *g*, *nd*, *rl*, *l*, and *bl* no linkage was observed. Not all possible

tests for linkage have yet been made. Cytological observations indicate that extensive linkage groups should not be expected, since the chromosomes are numerous (30 haploid) and are small short rods.

## SUMMARY OF MUTANTS

Table 1 summarizes the mutations. The information in the columns is: (1) culture number; (2) name of mutant; (3) symbol; (4) X-ray dosage; (5) sex of individual treated; (6) the generation after last treatment; (7) number and sex of individuals in which the abnormality was first noted.

TABLE 1

| (1)        | (2)                | (3)       | (4)  | (5) | (6)            | (7)     |
|------------|--------------------|-----------|------|-----|----------------|---------|
| M13/2b     | abnormal wing      | <i>g</i>  | 1800 | ♂   | F <sub>2</sub> | 1 ♂ 2 ♀ |
| M13/2g     | rolled scales      | <i>rl</i> | 2000 | ♂   | F <sub>2</sub> | 1 ♂     |
| M13/2h     | light body         | <i>l</i>  | 1800 | ♂   | F <sub>2</sub> | 1 ♂     |
| For/3M1    | light tegulae      | <i>lt</i> | 2200 | ♀   | F <sub>2</sub> | 3 ♀     |
| #115       | dark mesoscutellum | <i>ms</i> | ?    | ?   | ?              | 1 ♂ 1 ♀ |
| Ao3d/1a    | pink mesoscutum    | <i>me</i> | 1500 | ♀   | F <sub>2</sub> | 1 ♀     |
| For/g/1a   | red body           | <i>r</i>  | 2250 | ♀   | F <sub>2</sub> | 1 ♀     |
| M31/1b     | non-red body       | <i>nr</i> | 1200 | ♂   | F <sub>2</sub> | 4 ♂ 2 ♀ |
| M13/3b5    | eosin eye          | <i>e</i>  | 1800 | ♂   | F <sub>3</sub> | 1 ♂     |
| M13/5b543  | vermilion eye      | <i>v</i>  | 2200 | ♂s  | F <sub>2</sub> | 1 ♂     |
| M10/6c2511 | maroon eye         | <i>m</i>  | 2000 | ♀   | F <sub>3</sub> | 1 ♀     |
| F13/4b40   | shovel scale       | <i>s</i>  | 4500 | ♂   | F <sub>2</sub> | 4 ♂ 2 ♀ |
| TM11/5     | black body         | <i>bl</i> | 3500 | ♂   | F <sub>2</sub> | 1 ♂     |
| TM34       | needle scales      | <i>nd</i> | 4000 | ♂   | ?              | 1 ♂     |
| TM28       | chianti eye        | <i>ch</i> | 4000 | ♂   | ?              | 1 ♀     |
| TM17/21    | white eye          | <i>w</i>  | 4000 | ♂   | F <sub>2</sub> | 5 ♂ 3 ♀ |
| TM9/121    | white eye          | <i>w</i>  | 4000 | ♂   | F <sub>2</sub> | 2 ♂ 6 ♀ |
| TM28/22    | canyon eye         | <i>ca</i> | 4000 | ♂   | F <sub>2</sub> | 1 ♂     |
| TM2k/31    | salvia eye         | <i>sa</i> | 4000 | ♂   | F <sub>2</sub> | 3 ♂     |
| TM9/11     | rose of sharon eye | <i>rs</i> | 4000 | ♂   | F <sub>2</sub> | 2 ♂     |
| TM34/31    | jasper pink eye    | <i>ja</i> | 4000 | ♂   | F <sub>2</sub> | 2 ♂     |
| TM7/11     | rose leaf eye      | <i>rf</i> | 4500 | ♂   | F <sub>3</sub> | 1 ♂     |
| TM30/11    | reverse eye        | <i>re</i> | 4000 | ♂   | F <sub>3</sub> | 5 ♂     |
| TM7/42     | glassy eye         | <i>gl</i> | 4500 | ♂   | F <sub>2</sub> | 1 ♂     |
| TM17/43    | silvered eye       | <i>si</i> | 4000 | ♂   | F <sub>2</sub> | 2 ♂     |
| TM23/2     | spanish cedar eye  | <i>sc</i> | 4000 | ♂   | F <sub>2</sub> | 2 ♀     |
| TM23/2     | old gold eye.      | <i>go</i> | 4000 | ♂   | F <sub>2</sub> | 1 ♂     |
| TM23/2     | new gold eye       | <i>gn</i> | 4000 | ♂   | F <sub>2</sub> | 2 ♂ 3 ♀ |
| TM29/12    | yellow eye         | <i>y</i>  | 4000 | ♂   | F <sub>2</sub> | 1 ♀     |
| 13/6b3812  | lobed eye          | <i>lo</i> | 1800 | ♂   | F <sub>5</sub> | 1 ♂ 1 ♀ |
| TM20/2     | sorolla brown eye  | <i>sb</i> | 4000 | ♂   | F <sub>2</sub> | 2 ♀     |

## MOSAICS

From cultures of which one or both parents were heterozygous for the traits concerned, there have arisen a number of normal-mutant mosaics.

These mosaics uniformly show the mutant character in one lateral half of the body while the opposite half is normal. To date there have been detected thirty-four of these mosaic types, twenty males and fourteen females. These came from thirty-three cultures; in eight cases both parents were heterozygous and in twenty-five cases one parent was heterozygous and the other homozygous recessive.

The following is a list of mosaics with the culture and genotype of parents from which they came. The mosaic individuals are indicated with the characteristic of the left side above the line and that of the right side below the line, or graphically as if the individual's head were pointing to the right. (In handling and speaking of such mosaics it has become customary to hyphenate the mutant character name with "normal," using the word that indicates the condition of the left side first, as, "rolled-normal" or "normal-rolled.")

| CULTURE     | GENOTYPE OF PARENTS |              | SEX OF MOSAIC | MOSAIC   |
|-------------|---------------------|--------------|---------------|--|
|             | ♂                   | ♀            |               |  |
| 13/rc11     | <i>Rr</i>           | <i>rr</i>    | ♂             | $\frac{r\text{-body}}{\text{normal}} \rightarrow$    |
| 13/sb11     | <i>Gg</i>           | <i>gg</i>    | ♂             | $\frac{\text{normal}}{g\text{-wing}} \rightarrow$    |
| 13/4b31     | <i>gg</i>           | <i>Gg</i>    | ♂             | $\frac{g\text{-wing}}{\text{normal}} \rightarrow$    |
| 13/4c35     | <i>rr</i>           | <i>Rr</i>    | ♀             | $\frac{r\text{-body}}{\text{normal}} \rightarrow$    |
| 10/5c281    | <i>Rl rl</i>        | <i>rl rl</i> | ♂             | $\frac{\text{normal}}{rl\text{-scales}} \rightarrow$ |
| 13/4b32     | <i>Gg</i>           | <i>gg</i>    | ♀             | $\frac{g\text{-wing}}{\text{normal}} \rightarrow$    |
| Orig/6J1114 | <i>rl rl</i>        | <i>Rl rl</i> | ♂             | $\frac{\text{normal}}{rl\text{-scales}} \rightarrow$ |
| 10/5C271    | <i>Rl rl</i>        | <i>Rl rl</i> | ♂             | $\frac{rl\text{ scales}}{\text{normal}} \rightarrow$ |
| 13/6b3812   | <i>Lo lo</i>        | <i>Lo lo</i> | ♂             | $\frac{\text{normal}}{lo\text{-eye}} \rightarrow$    |
| P3/b21      | <i>Sb sb</i>        | <i>Sb sb</i> | ♂             | $\frac{sb\text{-cye}}{\text{normal}} \rightarrow$    |
| TM24        | <i>rl rl</i>        | <i>Rl rl</i> | ♂             | $\frac{\text{normal}}{rl\text{-scales}} \rightarrow$ |
| TM23/27     | <i>gg</i>           | <i>Gg</i>    | ♂             | $\frac{g\text{-wing}}{\text{normal}} \rightarrow$    |
| TM23/27     | <i>gg</i>           | <i>Gg</i>    | ♀             | $\frac{\text{normal}}{g\text{-wing}} \rightarrow$    |

# GENETICS OF GALLERIA

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| CULTURE               | GENOTYPE OF PARENTS |              | SEX OF MOSAIC | MOSAIC                                   |
|-----------------------|---------------------|--------------|---------------|--|
|                       | ♂                   | ♀            |               |  |
| TM <sub>23/26</sub>   | <i>Gg</i>           | <i>Gg</i>    | ♀             | $\frac{\text{normal}}{\text{g-wing}}$    |
| P-3/2110              | <i>ww</i>           | <i>Ww</i>    | ♂             | $\frac{\text{normal}}{w\text{-eye}}$     |
| TM <sub>25/3d</sub>   | <i>Sc sc</i>        | <i>sc sc</i> | ♂             | $\frac{sc\text{-eye}}{\text{normal}}$    |
| TM <sub>23/1</sub>    | <i>Gg</i>           | <i>gg</i>    | ♂             | $\frac{\text{g-wing}}{\text{normal}}$    |
| TM <sub>25/2</sub>    | <i>Rl rl</i>        | <i>Rl rl</i> | ♀             | $\frac{\text{normal}}{rl\text{-scales}}$ |
| TM <sub>21/1</sub>    | <i>Gg</i>           | <i>gg</i>    | ♂             | $\frac{\text{g-wing}}{\text{normal}}$    |
| TM <sub>14/2</sub>    | <i>Gg</i>           | <i>gg</i>    | ♀             | $\frac{\text{normal}}{\text{g-wing}}$    |
| TM-2                  | <i>Sc sc</i>        | <i>sc sc</i> | ♀             | $\frac{sc\text{-eye}}{\text{normal}}$    |
| TM-7                  | <i>G</i>            | <i>gg</i>    | ♂             | $\frac{\text{normal}}{\text{g-wing}}$    |
| P-8/21                | <i>sc sc</i>        | <i>Sc sc</i> | ♀             | $\frac{sc\text{-eye}}{\text{normal}}$    |
| TM <sub>26</sub>      | <i>rl rl</i>        | <i>Rl rl</i> | ♂             | $\frac{\text{normal}}{rl\text{-scales}}$ |
| TM-28                 | <i>Gg</i>           | <i>gg</i>    | ♂             | $\frac{\text{g-wing}}{\text{normal}}$    |
| TM <sub>23/11</sub>   | <i>gg</i>           | <i>Gg</i>    | ♀             | $\frac{\text{g-wing}}{\text{normal}}$    |
| TM <sub>23/21</sub>   | <i>gg</i>           | <i>Gg</i>    | ♀             | $\frac{\text{normal}}{\text{g-wing}}$    |
| TM <sub>28/313</sub>  | <i>sa sa</i>        | <i>Sa sa</i> | ♀             | $\frac{sa\text{-eye}}{\text{normal}}$    |
| TM <sub>23/27</sub>   | <i>Gg</i>           | <i>gg</i>    | ♂             | $\frac{\text{g-wing}}{\text{normal}}$    |
| TM <sub>28/2212</sub> | <i>Ca ca</i>        | <i>Ca ca</i> | ♀             | $\frac{ca\text{-eye}}{\text{normal}}$    |
| TM <sub>11/54</sub>   | <i>Gg</i>           | <i>gg</i>    | ♂             | $\frac{\text{normal}}{\text{g-wing}}$    |
| TM <sub>7/4111</sub>  | <i>Gl gl</i>        | <i>gl gl</i> | ♀             | $\frac{gl\text{-eye}}{\text{normal}}$    |
| TM <sub>23/r302</sub> | <i>Sc sc</i>        | <i>Sc sc</i> | ♀             | $\frac{\text{normal}}{sc\text{-eye}}$    |
| TM <sub>23/111</sub>  | <i>Ww</i>           | <i>Ww</i>    | ♂             | $\frac{\text{normal}}{w\text{-eye}}$     |



A few sex mosaics have appeared during this investigation. For the most part they have been of the bilateral type. In culture For/1a, the female parent of which had received 2,250 r units, there appeared a typical bilateral gynandromorph. On careful examination, it was found to be completely male in its left side in both coloration and form while the right side was typically and completely female, including its deeper pigmentation, its non-notched first wing, its forward projecting maxillary palp and its typically longer antenna. The male of the species is somewhat smaller than the female; and the body of this gynandromorph was flexed toward the left or male side. The line of demarcation between the two sex halves was distinctly shown by the dimorphic coloration of the body scales especially on the abdomen. An attempt was made to mate the individual to both a male and a female. Its behavior was more female than male in that it showed no aggressive tendency to copulate with a virgin female. However, a normal male copulated with it in a normal way except that the process was apparently imperfectly completed and was of shorter than normal duration. The animal seemed to have slightly less than normal vitality and became comatose about six days later. In the meantime it laid a total of 23 eggs, none of which developed. Its abdomen was removed at this point and given a critical examination. Externally, the left side had a normal male clasper and a diminutive adaegus while the right side lacked the clasper and had an ovipositor which was typical and apparently functional, but somewhat reduced in size. Internally, there was on the left side an incompletely formed bilobed testis with its ducts leading to the adaegus. On the right side was one complete ovary composed of its four normal ovarioles (egg chains) which took up most of the space in the abdominal cavity. The ovary had a normal oviduct which led into the ovipositor. The individual contained no recessive genes as markers, but an explanation will be suggested later as to its origin.

Among the offspring from a brother of the above gynandromorph mated to his sister there occurred another sex mosaic. The anterior end of this animal was typically male in that the maxillary palps hooked inward, the scales were lighter colored, and the antennae were shortened or male-like. The thorax and wings were typically female both in shape and coloration. The abdomen and external genitalia showed a combination of male and female characteristics. The left male clasper was about half normal size and the right only rudimentary. Between these projected an almost normal-sized ovipositor. Internally, there was no evidence of a testis and only a very rudimentary ovary.

Another culture produced a sex mosaic which had the characteristic coloration of a normal male. Its form, however, was typically female in

that its maxillary palps projected forward. It had straight wings without the indentation at the end which is characteristic of males, and its genitalia were normally female. The mother of this moth had been treated with 700 r units.

In a culture the male progenitor of which had been treated with 1,800 r units, there occurred a typically bilateral sex mosaic. In this case the right side was typically male, the left side female. The ovipositor, which was on the left side of the center of the last segment, was somewhat smaller than normal. On the right side was a normal male clasper. Internally, there was a slightly reduced ovary on the left side and a diminutive testis on the right.

Culture 13/7b38131 had one typically bilateral gynandromorph which lived only three days. Externally it resembled the one mentioned above except that its left side was male and its right side female. No internal dissection was made.

In culture TM23/25 was found a bilateral sex mosaic which was not detected until the second day after it had eclosed. During this time it had fluttered about until it had lost most of its scales. However, its left maxillary palp, left wing, and left clasper were typically male while the right palp, wing, and absence of clasper was female in structure. It had a diminutive ovary but no testis was found.

The mode of origin of the mosaics and gynandromorphs will be discussed below.

#### PARTHENOGENESIS

In a number of cases it has been shown that natural parthenogenesis obtains among certain species of moths (SEILER 1923 and 1929; HARRISON 1927; KOLTZOFF 1932, and others). YUNG (1925) makes brief mention of having isolated virgin females of *Galleria mellonella* and of having obtained impaternal offspring from them but he does not state the number or the sex of the offspring. He mentions that DONHOFF in 1858 isolated a newly eclosed female on a piece of wax and that three weeks later two larvae appeared. He does not state whether these two larvae completed their metamorphosis.

The fact that impaternal offspring have here been obtained from moths of this species with known mutant genes present as markers has thrown some light on the process of parthenogenesis.

In order to obtain females concerning whose virginity there could be no doubt, they were isolated as pupae. No means of distinguishing sex in the larval stage has yet been found. However, by carefully scrutinizing a great number of pupae a safe and dependable means of distinguishing their sex was found. Eighty-six normal female pupae were isolated. They were put, one each, into shell vials with pieces of sterile comb. The vials

were corked tightly with sterile corks and placed in the incubator at 30° C. These isolated females lived from 8 to 18 days, which is about their normal life. The vials were marked P-1 to P-86.

In nine of the eighty-six vials from one to 32 larvae appeared. Seven of the vials produced eclosed imagoes. The record of the nine vials is as follows:

| CULTURE | DAYS ♀<br>LIVED | NUMBER LARVAE<br>NOTED | NUMBER OF IMAGOEES ECLOSED |    |
|---------|-----------------|------------------------|----------------------------|----|
|         |                 |                        | ♂                          | ♀  |
| P-37    | 13              | 2                      |                            | 2  |
| P-38    | 14              | 1                      | 1                          | 0  |
| P-55    | 13              | 12                     | 1                          | 4  |
| P-60    | 9               | 2                      | 0                          | 1  |
| P-65    | 8               | 4                      | 3                          | 0  |
| P-68    | 18              | 1                      | 0                          | 0  |
| P-76    | 15              | 2                      | 0                          | 0  |
| P-77    | 11              | Many                   | 3                          | 5  |
| P-79    | 12              | 5                      | 2                          | 1  |
| Total   |                 |                        | 10                         | 13 |

The number of larvae noted was what could be seen through the vial and may represent fewer than were actually present. They were not removed from the vials and counted in order that any chance for contamination might be avoided. In culture P-76, the two larvae pupated but died in their pupa cases. The larva noted in P-68 reached full size but died without pupating.

This seemed to establish the fact that a small percentage of isolated virgin females do reproduce parthenogenetically. All these females were taken from normal wild stock.

In another series 114 virgin females were isolated. Seventy-seven were heterozygous for a mutant gene. Heterozygous individuals were used to determine whether any of the impaternal offspring were homozygous recessives and thus to throw light on the process by which parthenogenesis takes place in this species. In this series half-pint culture bottles were used. The bottles, food, and caps were sterilized. One virgin female was placed in each bottle. The bottles were labeled P-101 to P-214. In this series twelve females produced offspring. These cultures are listed below:

Although the data are meager, this second series of isolated females shows the very interesting fact that the heterozygous individual produces both wild type and recessive offspring. The impaternal offspring, both males and females, appear to be normal in every respect. Haploid individuals of a normally diploid species are not usually viable. If they live, their viability is greatly reduced. Since these *Galleria* appear completely normal, it is probable that they are diploid. The fact that the recessive types were

| CULTURE NUMBER | GENOTYPE OF FEMALE | NUMBER AND TYPE OF IMAGOS ECLOSED |         |
|----------------|--------------------|-----------------------------------|---------|
|                |                    | ♂                                 | ♀       |
| P-140          | +                  | 3+                                | 1+      |
| P-144          | +                  | 4+                                | 5+      |
| P-156          | <i>Gg</i>          | 1+                                |         |
| P-162          | +                  | 2+                                |         |
| P-166          | +                  |                                   | 1+      |
| P-169          | +                  | 7+                                | 5+      |
| P-180          | <i>Gg</i>          | 2+                                | 1+, 2gg |
| P-181          | <i>Gg</i>          | 1+                                | 2+      |
| P-184          | <i>Gg</i>          | 1+                                | 2+      |
| P-206          | <i>Ee</i>          | 1+, 1ee                           |         |
| P-211          | <i>Ee</i>          | 2+, 1ee                           | 2+      |
| P-212          | <i>Ee</i>          | 2+, 2ee                           | 2+, 3ee |
| Total          |                    | 30                                | 26      |

obtained only in the cultures where the mother was heterozygous for the mutant gene seems thoroughly to establish the occurrence of parthenogenesis and to rule out the possibility of accounting for the few progeny in each case as a result of contamination.

#### DISCUSSION

In addition to the discovery of many new and useful genes, one of the results of most interest was the detection of a number of individuals showing the effects of these mutations only in one half of the body. Not all of these mosaics could have arisen from unfertilized eggs because there are a few cases where the mother was recessive and one part of the mosaic showed the dominant allele from the male parent. The dominant character might be the result of somatic mutation but this explanation would involve the assumption of a high mutation frequency at a very early stage in the egg and no mutations at subsequent stages. Otherwise, there should occur mosaics with smaller fractions of the individual showing the dominant character. Since some of the mosaics come from fertilized eggs, they will all be considered to arise in the same manner unless later critical cases are found which require another explanation.

No mosaic thus far obtained has shown mosaicism for both sexual and mutant characters. Also, each mosaic has involved only one mutant character. As a result of this, critical evidence does not exist as yet to indicate with certainty the precise way in which gynandromorphs and somatic mosaics arise in *Galleria*.

In other species of Lepidoptera, there are a number of cases where mosaics may be best accounted for as arising from binucleate eggs. TOYAMA (1906) described a gynandromorphic silk worm larva which could be explained by double fertilization of a binucleate egg. DONCASTER (1914) demonstrated cytologically that binucleate eggs occur in *Abraxas* and that

each nucleus undergoes meiosis. WHITING (1922 and 1932) has found many mosaics in *Habrobracon*. He ascribes the origin of this mosaicism to egg binuclearity. His interpretation is that after the first meiotic division the nucleus of the second oöcyte gives rise to two pronuclei, that is, the second polar body is retained in the egg. In *Habrobracon* the males normally arise from unfertilized eggs and are haploid. If one of the two pronuclei of the binucleate egg is fertilized and the other develops parthenogenetically, either a haplo-diploid male mosaic or a gynandromorph is produced. Post-reductional binucleate (non-fertilized) eggs from heterozygous females produce impaternal male mosaics. WHITING states that egg binuclearity best explains the origin of mosaics in this parasitic wasp.

GOLDSCHMIDT and KATSUKI (1927) have demonstrated cytologically that egg binuclearity occurs in *Bombyx mori* and by the use of a mutant gene as a marker they obtained genetic evidence that led them to account for certain types of mosaics as arising by double fertilization of binucleate eggs.

It has been shown (GOLDSCHMIDT 1931) that in certain moths occasional eggs are binucleate and these may be doubly fertilized. GOLDSCHMIDT and KATSUKI (1927) found a recessive gene for the tendency toward sex mosaicism in *Bombyx mori*. They found these mosaics generally to be bilateral but frequently there were dorso-ventral types. Very infrequently irregular types occurred.

Egg binuclearity with appropriate double fertilization would explain the origin of sex mosaicism in *Galleria*. If the female, which is probably the heterogametic sex in *Galleria* as in other species of *Lepidoptera*, produces occasional binucleate eggs in which meiosis is post-reductional, then one of their nuclei would carry an X chromosome and the other a Y. And if in such eggs the nuclei were fertilized by sperm, all of which are X-bearing, they would produce bilateral (or dorso-ventral) sex mosaics. If on the other hand the meiosis had been pre-reductional and each of the two nuclei of the egg bore a Y chromosome, then double fertilization would produce a normal female. Likewise if the egg carried two X-bearing nuclei and these were fertilized by X-bearing sperm, a male would be produced. This condition of binucleate origin could be made detectable if each of the fusion nuclei had appropriate genes present as markers, in which case the individual would be a somatic mosaic. Such an individual could be simultaneously a gynandromorph.

The gynandromorphs have not appeared as frequently as the somatic mosaics. This would be the expected result if pre-reductional meiosis occurs more frequently for X and Y than for other factors. To date there has been no attempt to demonstrate cytologically egg binuclearity in this moth. However, the normal-mutant mosaics lend credence to the inter-

pretation that the binucleate condition obtains in a small percentage of the eggs.

While the results already obtained serve to demonstrate the occurrence of impaternal progeny, they are inadequate for a satisfactory analysis of the process of parthenogenesis in *Galleria*. The sex of the impaternal progeny is 40 males and 39 females which suggests that the mechanism of parthenogenesis must be one which will account for equal numbers of males and females. Where the mother was heterozygous, the progeny comprised 19 wild type and 9 autosomal recessive individuals. Since oögenesis has not been studied in *Galleria* it seems advisable to refrain from further speculation until more genetic and cytological information is available.

Isolated virgin females produced progeny in about one out of ten tested. This frequency appears to be higher than the frequency with which mated females produced either type of mosaic. No evidence has been found which indicates that impaternal moths are produced from mated mothers.

#### ACKNOWLEDGMENTS

The work was done under the supervision of Professor D. E. LANCEFIELD. Thanks are also due the Crocker Research Laboratory, New York City, and the Carnegie Institution, Department of Genetics, at Cold Spring Harbor, New York, for their kind permission to use their X-ray machines. The original wild stock was obtained from Professor IVAN R. TAYLOR of Brown University. Acknowledgments and thanks are due the National Research Council and the Sigma Xi Society for grants-in-aid which have made the continuation of the research possible.

#### SUMMARY

1. Moths of the species *Galleria mellonella* are well adapted to laboratory conditions in that they are viable, easily cultured, prolific, and breed the year round.
2. The species seems to be very stable genetically in that no mutation has been detected in untreated stocks.
3. X-rays are effective in producing mutations in the normal wild species.
4. Irradiation in the adult stage has proved most effective for producing mutations.
5. The mutations found act as autosomal recessives.
6. Thirty-one mutants are described from among some two hundred abnormalities which appeared after irradiation.
7. One case of linkage has been found between the genes for light body color and rolled scales, with about 2 percent crossing over.

8. Crossing over has been observed only in males.
9. The chromosomes are very small and numerous—typically lepidopteran. The diploid number is 60.
10. Heterozygotes backcrossed to recessives produce occasional mutant-normal mosaics. These may arise from appropriate double fertilization of binucleate eggs.
11. Infrequently gynandromorphs have been detected. They may arise from an X- and Y-bearing binucleate egg fertilized by two X-bearing sperm.
12. Facultative parthenogenesis has been observed to occur in about 10 percent of the isolated virgin females.
13. The impaternal offspring are of both sexes in approximately equal numbers and are presumably diploid.
14. Isolated virgin females, heterozygous for a recessive trait, produce both the recessive and the dominant phenotypes.

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# ABSTRACTS OF PAPERS PRESENTED AT THE 1937 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

WOODS HOLE, MASSACHUSETTS, AUGUST 30-SEPTEMBER 1, 1937

INDIANAPOLIS, INDIANA, DECEMBER 28-30, 1937

M. DEMEREC, *Secretary*  
*Carnegie Institution of Washington*  
*Cold Spring Harbor, New York*

BANTA, A. M., and WOOD, THELMA R., Brown University and Carnegie Institution of Washington, Providence, R. I.: *Maternal effects in Cladocera*.—On analysis three independent groups of data show results readily explicable as maternal effects. Sex intergradedness and excavated head in *Daphnia longispina* are characters highly variable in their phenotypic expression within a clone. (1) The average degree of sex intergradedness of offspring from high mothers of a clone is nearly the same as that for simultaneously reared offspring of low mothers of the same clone; but in each of the series tested the high mothers had a slightly higher average expression of sex intergradedness in their offspring than did the low mothers. (2) In certain of the selection experiments involving the excavated head character the high selections for a long period of parthenogenetic generations produced offspring which had means slightly but consistently above the corresponding means for the low selected line of the same clone; yet these differences were apparently non-genetic. These significant data, obtained late in the selection experiments, seem highly reliable. (3) In sexual reproduction, ♀ from sex intergrade clone by ♂ from normal clone, as compared with the reciprocal cross (♀ normal by ♂ sex intergrade), produced offspring clones averaging higher in their expression of sex intergradedness. The same applies to reciprocal crosses of excavated head by normal.

BELGOVSKY, M. L., and MULLER, H. J., Institute of Genetics of the Academy of Sciences of the U.S.S.R., Moscow, U.S.S.R.: *Further evidence of the prevalence of minute rearrangement and absence of simple breakage in and near chromocentral regions, and its bearing on the mechanisms of mosaicism and rearrangement*.—Other X chromosomes constituted like scute-8, for example scute-S1, give similar results. In Bar-M2, having an interstitial chromocentral region slightly proximal to forked, absence of simple breaks is phenotypically evident, but irradiation produces comparatively many forked "mutations," often with lethals, mosaicism, etc., illustrating predisposition of chromocentral and any adjoining regions to compound breakage with minute rearrangement. —Supposing the spontaneous tendency to such rearrangement intensified in somatic cells, these results lead to interpretation of "eversporting displacements" essentially like SCHULTZ's recent interpretation based on his salivary finding of minute deletions. (Conceivably, additional chromocentral sections,

by conjugating, might, like splints, hinder rearrangement.)—The frequencies and frequency-dosage relations of these rearrangements indicate that the simultaneous neighboring breaks are interdependent, and hence secondary consequences of some primary chemical change occurring outside the parts broken.

BERGER, C. A., Woodstock College, Woodstock, Md.: *Prophase pairing in somatic cells with multiple chromosome complexes.*—The diploid chromosome number of *Culex* is six. During metamorphosis, a series of multiples of six: 192, 96, 48, 24 and 12 chromosomes, are found in dividing epithelial cells of the ileum. Prophase stages of cells with high multiple complexes show six groups of sister threads. The members of each group are closely associated in a cable-like structure. Homologous groups are loosely paired. Prophase groups containing 8, 16 or 32 chromonemata in close, side-by-side association, furnish an interesting norm of comparison for studies of the structure of giant gland chromosomes. The association of so many sister threads seems clearly to show that the attraction between like chromosomes is not satisfied by the union of two (DARLINGTON). The presence of six groups instead of three gives evidence of a primary attraction between sister chromosomes and a weaker secondary attraction between homologous groups.

BISHOP, MAYDELLE, University of Texas, Austin, Tex.: *X chromosome duplications in Drosophila melanogaster.*—Among 13,017 F<sub>1</sub> females, obtained by X-raying normal males and mating to *y v f car* attached-X females, 154 were hyperploid for part of an X chromosome. These were: *v f car* 105, *f car* 4, *car* 1, *v f* 14, *f* 6, *v* 12, *v car* 1, *y v f* 8, *y v* 1, *y* 2. Of these, 143 resulted from the deletion of the middle of the X, leaving the reunited left and right fragments, and 11 from simple breaks, or deletions with the left break to the left of *y* (o.o). The *v car* female is an example of a double deletion. The *f-car* sector of the X is slightly longer than the *car-bb* sector both in crossover units and in the salivary gland chromosome. Females hyperploid for this XR section of the chromosome (*B-bb* sector) are as viable as normal females (PATTERSON, STONE, BEDICHEK). Nevertheless comparable duplications (from deletion) involving these two sections are not of equal frequency. In all, 109 involved the *car-bb* interval and 20 the *f-car* interval, again illustrating that the *car*-centromere interval is differentially susceptible to breakage. XR fragments which result from "simple" breaks have the advantage of viability. Nine occurred as compared with 26 comparable deletions. If chromosomes with a "broken end" from simple breaks can survive in *D. melanogaster*, deletions, being more numerous, cannot be regarded as the result of two unrelated chromosome breaks.

BLAKESLEE, A. F., AVERY, A. G., and CARTLEDGE, J. L., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Induction of polyploids in Datura and other plants by treatment with colchicine.*—A number of chemicals

have been tested with *Datura* and other plants in an attempt to induce hereditary mutations. Among these, chloral hydrate, earlier used to induce chromosome doubling in roots, failed to cause doubling in shoots though purple pigment in seedlings from treated seeds was temporarily inhibited. Colchicine induces abundant production of branches in which the number of chromosomes is doubled. When seeds are heavily treated, plumule growth is checked, shoots develop from axils of cotyledons, buds in stems are abnormally arranged leading to sectors with roughened leaves characteristic of mixed  $4n$  and  $2n$  tissue like spontaneous  $4n$  sectorial mutations. Normal  $2n$  tissue tends to outgrow the mutated  $4n$  tissue, but the latter may apparently include the whole shoot. Tetraploid tissue involving the flower may readily be determined by examination of pollen. Methods of treating adult tissue are being investigated. If control of chromosome doubling by chemical means proves of general application (for which there is already some evidence) the plant breeder will be able to work with greater precision in his efforts to control evolution of economic forms, both of plants propagated by vegetative methods and of those reproduced by seed. For example, it should be possible, starting with a sterile hybrid, to synthesize a pure-breeding double diploid which would have hybrid vigor and the desirable characteristics caused by tetraploidy. Tetraploidy would give enlarged flowers and fruits to the horticulturalist and be the basis through triploids of a wide range of  $2n+1$  chromosomal types.

BLANC, R., and CHILD, G. P., Amherst College, Amherst, Mass.: *The effect of high temperature on the "truncate reaction" in *Drosophila melanogaster**.—Larvae and pupae of non-dumpy and heterozygous dumpy flies were exposed to  $36.5^{\circ}$  for 12 hours during different periods of development. Many "heated" flies showed wing and thorax characters simulating the expression of dumpy and/or truncate. The effect was much more pronounced in the heterozygous dumpy stock than in the non-dumpy stocks. The temperature-effective period for this truncate reaction was found to occur during the first day of pupal life in all the stocks used. By removing timed pupae and subjecting them to the heat treatment at definite periods after pupation, it was found that the effective period was more closely related to pupal age (physiological age) than to age from egg-laying (total chronological age). Males showed a greater effect than females with respect to both number and degree of the truncates.

BOGART, RALPH, SMITH, S. E., and KIMBALL, GRACE, Cornell University, Ithaca, N. Y.: *Anemia, a recessive lethal in the rat*.—The anemic rats appear normal at birth, but develop a yellowish color when 3 to 5 days old. This yellow color becomes more marked with age, and growth is retarded. Death occurs at about 2 weeks of age, and at that time there is much difference in the size and appearance of these animals as compared with the normal litter mates.—The average haemoglobin value for the anemic rats is 4 gms per 100 ml of blood and for the normal sibs, 9 gms. Red cell counts are low, being about one-third those of the normal litter mates. Bilirubin is present in the

blood sera of all the anemic rats that have been examined, but none was found in the normal rats (VAN DEN BORGH). The heterozygotes are entirely normal. Free iron is present in large amounts in the liver of the anemic rats, but such quantities are not found in the liver of the normal sibs.—Genetic studies indicate that this condition is due to a simple recessive. Matings of male or female normal litter mates of the anemics to known carriers show that about two-thirds of the litter mates carry the gene, while one-third are homozygous normal. When animals known to be carriers of this gene were mated together, the result was 246 normals and 72 anemics. This is a good fit to a 3 to 1 ratio. The gene is not sex linked.—The anemic animals have not responded to iron-copper or liver therapy. (This study was supported by funds from the Rockefeller Foundation Grant for Research in Longevity.)

BREHME, KATHERINE S., Columbia University, New York, N. Y.: *The time of death of three Minute homozygotes in Drosophila melanogaster*.—As a clue to the mechanism of the Minute reaction, the lethal action of the homozygous *Mw*, *MFla*, and *MI<sup>2</sup>* factors is being studied. Egg, larval, pupal and adult counts of offspring from crosses of Minute males by Minute females show that the homozygous Minutes die as larvae. Observation of Petri dish cultures show that all Minute homozygotes die in the first larval instar; such larvae may be recognized by their sluggish, flaccid appearance. A few lethal larvae survive two days after hatching (25°C) but do not undergo the first moult although their sibs are then in the third or late second instar.

BREHME, KATHERINE S., Columbia University, New York, N. Y.: *The growth curve of Minute larvae*.—Measurements of larvae from crosses of *Mw* and *MI<sup>2</sup>* males by wild type females show, at 24, 48 and 96 hours after oviposition at 25°C, a unimodal frequency distribution of larval length. Measurements of *Mw* populations just before pupation of the wild type (100 hours), and when all wild type larvae have pupated and Minute larvae are beginning pupation (144 hours), indicate that *Mw* larvae do not increase significantly in length during the two days of delay. At 25°, therefore, these Minute heterozygotes follow the same curve of growth in larval length as the wild type, entering upon a growth plateau at pupation of wild type. As Minute imagoes are smaller than non-Minutes, it is suggested that the Minute reaction is not effective upon growth of larval tissue but upon imaginal tissue through an effect on cell division.

BRIDGES, C. B., Carnegie Institution of Washington and California Institute of Technology, Pasadena, Cal.: *Revision of the salivary map of the X chromosome of Drosophila melanogaster*.—For precise localization within the X chromosome of the points of breakage of aberrations, and for determining accurately the relation between crossing-over and the chromosome axis, more reliable salivary maps are required than those already available (PAINTER 1934, BRIDGES 1935). Accordingly, very careful drawings at 5000 diameters

were made for each of the twenty divisions of the X chromosome, using only permanent preparations especially favorable in fixation, staining, size and non-kinked stretch of the chromosomes. Averaged drawings were made from the best drawings (3-7) for each section. The composite map is 414 microns long, as compared with 220 microns for the 1935 map, and approximately 1024 lines are recognized as compared with 725. The relative intensity, spacing and characteristics of the lines are much better represented. A feature of great convenience of the new map is the numbering of all lines, hence references to position are precise.

CAMERON, JOHN A., Harvard University, Boston, Mass.: *Hair color changes in mice as indicators of the spread of X-ray effects.*—New hair produced by black or agouti mice, on areas depilated by X-ray exposure, is white or silver colored. The white areas exactly conform to the area of exposure while the hair on lead-shielded areas retains the original color. Mice whose posterior halves have been turned white by moderate X-ray exposure exhibit sharply defined boundaries after many months.—Some mice, exposed to sub-lethal X-ray dosage, become white first on the exposed areas and later, by progressive extension from the exposed area forward, white over the entire body. This change occupies five to six months.—It is suggested that this is a cell to cell transfer of substances upsetting the enzyme-pigment balance of the cells concerned and that it represents a transfer of X-ray effect into a lead shielded region.

CARLSON, J. GORDON, University of Alabama, Tuscaloosa, Ala. and Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Some effects of X-radiation on somatic chromosomes of Chortophaga viridifasciata.*—Neuroblast chromosomes of irradiated grasshopper embryos show in succeeding stages of the same mitotic cycle the following deviations from untreated material: 1) Fragmentation. The distal fragments, which lack spindle fiber attachments, are arranged irregularly outside the metaphase spindle. Chromatids of such distal fragments separate either entirely to form two rods or partly to form V's or rings. At telophase these fragments become diffuse in "accessory nuclei" outside the main nucleus. 2) Translocations. U-shaped chromosomes at metaphase and U-shaped chromatids at anaphase with spindle attachment loci at both ends prove that attachments have occurred between different chromosomes, presumably in connection with loss of the distal ends by fragmentation. 3) Chromosomal constrictions in unusually large numbers may represent places of incomplete fragmentation and/or places of reattachment following fragmentation. 4) Chromatid inequalities are present as: non-terminal chromosome fusions resulting from single chromatid lesions in two or more chromosomes with reattachment of non-sister chromatids; unequal metaphase chromatids; and anaphase sister chromatids of unequal length or with non-corresponding constrictions. 5) Half-chromatid inequalities are suggested by minute fragments and by regions in the anaphase

chromatid of abnormally small diameter.—These observations indicate that 1) chromosomes treated with X-rays are fragmented and translocated during or immediately following irradiation, 2) X-rays can affect either one or both chromatids of a chromosome, and 3) interphase and prophase chromatids probably consist of more than one chromonema.

CHASE, HERMAN B., University of Chicago, Chicago, Ill.: *Biometric study of white spotting in the guinea pig*.—The strain (35 D) used in this study traced to one mating in the 22nd generation of brother-sister mating. There was variability about the average (70 percent white) from a trace to 100 percent white (nongenetic as indicated by parent-offspring correlation,  $+0.026$ ). Presence of color was determined in each of 30 points on drawings of the 405 animals. All of the possible 276 tetrachoric correlations were calculated among the 24 points with from 10 percent to 81 percent color. Forehead and eye points show rather low correlations even with each other. Apart from these, the points on each side fall into 3 groups within which there are high correlations ( $+0.60$  to  $+0.96$ ) but between which the correlations average much lower. Correlations are higher across the midline of back (average  $+0.47$ ) than across the mid-belly (average  $+0.21$ ). The dorsal points show their highest correlations with points ventrad ( $+0.80$ ) or anteroventrad ( $+0.76$ ). Embryonic proportions offer a possible explanation. The cheek-ear-shoulder region shows practically no correlation with loin-rump-hind leg region of the opposite side (18 correlations, average  $+0.04$ ) and a significantly negative correlation with this region of same side (18 correlations, average  $-0.08$ ). Corroborative evidence has been obtained from another inbred strain. Absence of positive correlations between remote points indicates that the nongenetic factors do not act much on the animal as a whole. This is in harmony with the low correlation between littermates ( $+0.115$ ). Negative correlations between certain regions on the same side suggest interference.

CLELAND, RALPH E., Goucher College, Baltimore, Md.: *The present status with regard to segmental arrangements in Oenothera*.—The author is engaged, in collaboration with Dr. P. A. MUNZ, in a study of the wild *Oenotheras* (subgenus *Onagra*) throughout their entire range. The segmental arrangements are being determined of as many as possible of these forms, it being hoped that a comparison of the various complexes from the standpoint of their segmental arrangements will make possible, in conjunction with taxonomic criteria, an understanding of the nature of the relationships between the species, and of the forces which have been of predominant importance in their evolution. The occasional occurrence of segmental interchange in our own experimental material has rendered definitive analyses difficult, but considerable progress has been made. Formulae for 8 new complexes have been tentatively determined, and the possibilities for a number of other complexes have been reduced.

COLLINS, J. L., and KERNS, K. R., University of Hawaii, Honolulu, T. H.: *Mutations in the pineapple, Ananas comosus (L) Merr.*—The Cayenne variety has been propagated asexually for 100 years. It now has an extremely heterozygous genotype and is composed of a number of clones. Heterozygosity has undoubtedly increased during this period of vegetative reproduction through the occurrence of mutations. Mutations have appeared influencing plant, inflorescence and fruit characters and which indicate something of the type of characters potentially present in the genotype as heterozygous recessives. The rate of mutation is generally low but the number of plants growing annually, approximately 1,800,000,000 shoot meristem areas, gives opportunity for the appearance of many mutations. The vegetative method of propagation preserves and increases the number of mutant individuals. Mendelian analysis has shown that both simple gene mutations and those of a more complex nature have occurred including dominant, recessive and lethal changes.

COLLINS, J. L., and KERNS, K. R., University of Hawaii, Honolulu, T. H.: *The origin and breeding characteristics of polyploid pineapples, Ananas comosus (L) Merr.*—The majority of pineapple species and varieties have the diploid number of 50 chromosomes. Triploid and tetraploid forms containing 75 and 100 chromosomes occur infrequently in  $2N$  hybrid populations. Triploids originated by fertilization of  $2N$  eggs and normal  $N$  pollen. One  $4N$  originated as a single plant in a varietal hybrid population. This varietal amphidiploid could have originated either from the union of two  $2N$  gametes at fertilization or through zygote doubling following fertilization of  $N$  gametes. Other  $4N$  plants were the entire progeny of a  $2N \text{ } \varnothing$  by  $3N \text{ } \sigma^7$  cross and resulted from the fertilization of a  $2N$  egg gamete from the diploid parent by  $2N$  pollen gametes from the triploid parent. No  $3N$  plants were produced in this cross. Meiosis in triploids is very irregular and very few functional gametes are produced. Tetraploids have a regular meiosis and produce a high percentage of normal gametes. Crosses of  $2N$  by  $4N$  have failed to produce the expected large numbers of  $3N$  forms. The majority of the progeny from such crosses are  $4N$  with a few  $3N$  and  $2N$  plants.

CREIGHTON, MARGARET, State University of Iowa, Iowa City, I.: *Parthenogenesis in Chorthippus curtipennis.*—In a project on the genetics of this species, isolated virgin females have been found to lay unfertilized eggs within about two weeks after the final moult. Unfertilized eggs are very poorly viable, only a few ever reaching the hatching stage. Of those that hatch, nearly all die within a few hours. Some fail to accomplish the first moult at hatching. Monsters of various kinds are frequently formed and usually lead to the early death of the embryo. Unfertilized eggs undergo both maturation divisions in the same manner as fertilized eggs. Embryos examined at later stages, however, show both haploid and diploid cells. The mechanism by which the change from haploid to diploid takes place has not yet been worked out but further investigation is in progress on this point.



CUMLEY, R. W., University of Texas, Austin, Tex.: *Serology of Drosophila*.—Rabbits were inoculated with saline extracts of various *Drosophila* species. The antisera so produced were tested with complement fixation and precipitation technics. In this way the antigenic relations of several species of *Drosophila* were established. Results of these tests were compared with data regarding the taxonomic relationships of the species in question. The taxonomic and serologic relationships were found to be similar.

CUMLEY, R. W., University of Texas, Austin, Tex.: *Effect of temperature on fertility and viability of hyperploid males of Drosophila melanogaster*.—Males of *D. melanogaster* carrying in duplicate the X chromosome regions 8A through 9A, 0-through 3C2, or 18D through 20D (BRIDGES' 1935 map) were raised at 13.5°, 17.5°, 21°, 26°, and 29°C. These three particular hyperploid stocks were selected because, at normal temperatures, they have quite different fertility and viability. A  $\gamma^2$  control was carried at the same temperatures. All males were tested for fertility and viability by mating to  $\gamma$  *vf* attached-X females. Control  $\gamma^2$  males and 8A-9A males were most fertile if raised at 17.5°-21°C; 0-3C2 males were most fertile if raised at 17.5°C.; and 18D-20D males were most fertile if raised at 21°C. The two latter groups had relatively small ranges of tolerance to temperature, and were almost completely inviable at 13.5°C. The 8A-9A duplication males had viabilities more nearly normal.

DAVENPORT, CHAS. B., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *How family resemblance of features is brought about*.—Profiles of two unrelated grown boys with unlike facial features are shown. Also the changes in proportions of these features are traced year by year from an early age. The adult differences are largely foreshadowed in the child. In contrast is shown the profile of a pair of identical twin girls. The growth changes experienced by their features are extremely similar. The end result is great facial similarity. If in the latter case the similarity of growth of features is to be ascribed to the similar action of like genes, in the first case the unlikeness is to be ascribed to dissimilar genes guiding development. In view of the great range of kinds of facial features in mankind, the number of kinds or modification of genes affecting facial features must be great, even if we assume that a large part of the difference between features is due to dissimilar combinations of relatively fewer genes.

DUNN, L. C., and GLÜCKSOHN-SCHOENHEIMER, S., Columbia University, New York, N. Y.: *A dominant short-tail mutation in the house-mouse with recessive lethal effect*.—Short-tailed mice found by Prof. C. H. DANFORTH among descendants of his posterior duplication stock have been tested and found to contain a new dominant. Heterozygotes resemble the Brachury mutant type but the tails are somewhat shorter, varying from tailless to about  $\frac{1}{3}$  of normal. Homozygotes are entirely tailless, have a marked lesion in the sacral region resembling spina bifida, have no anal opening and generally no

genital papilla. These invariably die within 24 hours after birth. Matings of short by short have given 90 short, 35 normal and 41 of the lethal type. Short by normal matings have given 124 short, 109 normal.—The new mutant (known as D-short, *Sd*) has been tested for allelism with Brachury, *T*.  $F_1$  consisted of 29 Brachy, 24 D-short, 41 tailless, 31 normal.  $F_1$  tailless when tested by normal gave about equal numbers of Brachy, D-short, tailless and normal (54:44:45:46). This shows that animals heterozygous for both *T* and *Sd* are tailless, and that *T* and *Sd* assort independently. The effects of these non-allelic tail mutations are therefore cumulative.

EYSTER, H. CLYDE, Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Natural mutation rate in corn*.—The natural mutation rate in corn is much higher than most geneticists suppose. In a commercial field of corn in Central Pennsylvania 73 virescent seedlings were found among a calculated total of 6510 seedlings. These 1.12 percent of seedlings which were virescent were albino in nature with a small amount of chlorophyll near the tips of the leaves, and lived only about 3 weeks at the most. Theoretically lethal recessive mutants tend to disappear from a freely self and cross pollinated population such as corn. Hence, the mutation rate of this virescent gene and possibly numerous other loci must be rather frequent. The interaction of natural selection and of the mutation rate controls the number of double recessive mutants appearing in a heterogenous population of corn.—Two years ago the author in collaboration with Dr. WILLIAM H. EYSTER reported at the St. Louis meetings 40 spontaneous gene variations which occurred naturally from a commercial strain of corn and which affected every part of the plant in almost every way that is possible. Due to a high natural mutation rate in corn it is absolutely necessary to have controls in experiments so as to differentiate between naturally occurring and artificially produced changes.

GABLE, JEANNE V., New York University, New York, N. Y.: *Temperature effects on the wings of vestigial-No wing (*vg*<sup>33/13</sup>)*.—The mean lengths and areas of the wings of vestigial-No wing/wild from matings of such heterozygotes vary inversely with the temperature, as do those of wild/wild, but are significantly lower in value. The phenotype is nicked or notched with the lateral margins usually complete. It occasionally overlaps wild type. The wings of vestigial-No wing flies sired by homozygous vestigial-No wing out of vestigial-No wing/wild females show very deep V-shaped terminal notches instead of the shallow notches in the wings of the heterozygotes from heterozygous parents. The wings of vestigial-No wing/vestigial-pennant vary inversely in size with the temperature, the phenotype ranging from "strap" to "vestigial" with the rise in temperature. The wings are carried in the normal position back over the abdomen or only at a slight angle to the longitudinal axis of the body. The wings of vestigial-No wing/vestigial flies are indistinguishable from those typical of homozygous vestigial but in contrast to homozygous vestigial they remain "vestigial" in appearance through the viable temperature

range. The wings of homozygous vestigial-No wing flies are "apterous" in phenotype and do not vary with the temperature.

GOODALE, H. D., Mount Hope Farm, Williamstown, Mass.: *Measuring the value of breeding methods in improving livestock*.—The value of different methods of breeding in improving live stock can be measured by comparing the rate of change in the average which takes place under one method of breeding with the rate under other methods. Charts showing the rates of change obtained by the use of the family method (progeny of one sire and dam), as it is called at Mount Hope, on a number of characters in different kinds of animals will be exhibited together with charts showing results obtained by modifications of this method and by other methods. Specimens, some living, showing the amount of change will also be shown.

GOODSPEED, T. H., and MALLOCH, W. S., University of California, Berkeley, Cal.: *Temperature, X-ray and neutron studies on Neurospora*.—A temperature of 59°–61°C, in a thermostatically controlled water bath, is favorable for germination of ascospores and for destruction of conidiospores or mycelial growth on inoculated agar slants. One and one-half hours of treatment is required for *N. tetrasperma*, but two hours are favorable for germination of ascospores in *N. sitophila*. Exposure of conidiospores or mycelia to shorter heat treatments at 59°–61°C results in variation.—With low humidity, at a temperature of 25°C, irradiated ascospores of *N. tetrasperma*, upon germination, exhibit an increase in the number of variant cultures with each progressive increase in the length of the storage period.—Storage of untreated ascospores at 42°C induces variation in the resulting cultures, while in irradiated cultures the alterations due to heat and X-radiation become cumulative. At 0°C the untreated cultures are apparently uninjured, but the irradiated cultures show a cumulative effect.—Ascospores treated with Grenz rays produce a large number of variant cultures in *N. tetrasperma*. Neutrons produce an equivalent effect at a lower "dosage." In both cases percentage of abnormal forms increases with an increase in dosage. *N. sitophila* is more resistant to Grenz radiation injury than *N. tetrasperma*; the effect on the former is shown by a shift in the ratio of cultures with conidiospores to cultures without conidiospores in a hybrid involving these characters.—Several degrees of retardation of development occurred in derivatives of X-rayed ascospores. Early growth of certain cultures corresponds with that of unisexual dwarfs, but later on their bisexual nature is apparent by their production of perithecia and ascospores. Certain bisexual X-ray derivatives cross with either sex as a result of selective pairing in the ascus hook.

GOWEN, JOHN W., Iowa State College, Ames, Ia.: *A comparison of X-ray inactivation rates in Drosophila and in tobacco mosaic virus*.—Data showing the inactivation rates of different *Drosophila* cells and tobacco mosaic viruses will be compared. These data bring out similarities of these X-ray effects in gene and virus particles.

GREEN, E. L., Brown University, Providence, R. I.: *The inheritance of coastal and vertebral variations in the rabbit*.—Two selected and inbred families of rabbits differing with respect to the number of pairs of ribs and vertebrae were crossed to produce two hybrid and two back-cross generations. The normal family is practically true breeding for twelve thoracic and seven lumbar vertebrae. The 13-ribbed family is relatively constant in the occurrence of extra ribs but somewhat variable in the degree of development of the ribs and in the number of lumbar vertebrae, which may be six or seven. In this cross the mere presence of extra ribs, without regard to their expressivity, is apparently a Mendelian recessive, as indicated by their suppression in  $F_1$  and by their recovery in expected monofactorial frequencies in succeeding generations. In the 13-ribbed family complete development of the extra ribs is generally associated with seven lumbar vertebrae; incomplete development, with six. This association may be caused by variable action of a growth factor whose effect extends throughout the vertebral region including the thoracolumbar and lumbo-sacral borders. However, the fact that the  $F_2$  and back-cross animals have ribs less completely developed on the average than the 13-ribbed parents, and that six lumbar vertebrae occur more frequently than seven indicates that there are also genetic modifiers of the expressivity, some of which were introduced by the 12-ribbed parent. Additional breeding tests are designed to test the validity of these conclusions and to explain the production of a few 12-ribbed animals in the 13-ribbed family.

GRIFFEN, A. B., and STONE, W. S., University of Texas, Austin, Tex.: *Gene position and mottling*.—Two mutual translocations involving the X chromosome, each broken to the right of line 3C2 (BRIDGES' 1935 map) produce mottled eyes. These white mottles are  $w^{M5}$  and 609e.  $w^{M5}$  has moved XL including 3C2 to a position next 101F3 of chromosome IV. 609e has attached XL through 3C2 next to 100C3 in chromosome III. Irradiation has induced several changes of these mottled phenotypes. In the case of  $w^{M5}$ , fifteen cases of phenotypic change were accompanied by gene rearrangements involving XL through 3C2. The new phenotypes: ten cases have red eyes with black flecks, one lemon with vermilion spots, one white (only break to left of 3C2), and three normal red which on irradiation produced secondary mottles that involved further rearrangements. Mottled 609e on similar treatment changed often to a lighter color and several times to a darker color (with and without mottling). The darker changes involved gene rearrangements. Several Plum alleles (SUCHE) and  $w^{M4}$  (an inversion, analysis incomplete) have also reverted to normal phenotype. Obviously the normal red condition is independent of the old association of genes in the X chromosome; mottling at the white locus is likewise independent of the original translocations and of juxtaposition to the chromocenter.

GUSTAFSSON, ÅKE, Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Species formation and polyploidy within the apomictic genera Rubus*

and *Taraxacum*.—All American species belonging to *Rubus* subgenus *Eubatus* are sexual, even those closely related to the European blackberries. The thousands of European microspecies, published by taxonomists, belong to two different groups: *Rubi Eubati veri* and *Rubi Corylifolii*. Only two sexual diploid species are known. All more or less apomictic *E. veri* are triploid, tetraploid or pentaploid ( $3x=21$ ,  $4x=28$ ,  $5x=35$ ). *Rubi Corylifolii* are hybrids between *E. veri* and the tetraploid apomictic *R. caesi*us. Their chromosome numbers are  $4x=28$ ,  $5x=35$ ,  $6x=42$  and  $7x=49$ . All microspecies can be grouped into six classes of species with a different taxonomical value, but, due to panmixis, the taxonomy of the subgenus is arbitrary and superficial. Only at the outskirts of their distribution area, the depauperated biotype-compounds can be divided into groups, the limitation of which probably is phylogenetically determined.—The North European apomicts of *Taraxacum* belong to six different groups, which may be called agamospecies (TURESSON). They are taxonomically and ecologically well limited. The apomicts of the group *Vulgaria*, containing more than 300 published species, are triploid ( $3x=24$ ) throughout. Last year, however, a sexual diploid population with a limited distribution also belonging to *Vulgaria* was found. *Spectabilia* is an atlantic tetraploid group ( $4x=32$ ). *Ceratophora* consists of arctic tetraploid apomicts, *Palustria* of tetraploid apomicts limited to swampy localities, and *Erythrosperma* and *Obliqua* form two lime or sand groups with triploid as well as tetraploid apomicts.—Hence a rather marked difference exists between the facultative apomicts within the genus *Rubus* and the obligatory apomicts in the genus *Taraxacum*. In *Rubus* the biotypes do not group themselves into natural units, whereas this more or less is the case within *Taraxacum*.

GUYER, M. F., MOHS, F. E., and CLAUS, P. E., University of Wisconsin, Madison, Wis.: *Inheritance of resistance to transplantable cancer in rats*.—By selective breeding through thirteen generations a strain of rats highly resistant to implants of the Flexner-Jobling rat carcinoma (FRC) was isolated from a very susceptible stock which in transplants to 1543 individuals has shown 1303 "takes," or 84.4 percent. Susceptibility was reduced in the selected strain in the 8th to 13th generations to 6 percent of "takes" (62 takes out of 1043 implanted individuals). In crosses of the immune strain with the unselected stock the total  $F_1$  hybrid generation showed 87 percent of "takes" (75 individuals out of 96 implanted) compared with 77.6 percent (52 individuals out of 67 implanted) in controls from the original unselected population. Backcrosses with each parent type and interbreeding of the  $F_1$  hybrids yield progeny which indicate that far fewer dominant factors for susceptibility are involved than the number indicated in similar cross-breeding experiments with mice, in which, however, the several experimenters used widely different strains instead of strains which are probably very closely related except in the matter of susceptibility to cancer implants. The total numbers of progeny in our later generations are still too few to draw trustworthy conclusions. The

main interest of the work lies in the possibility of determining something about the mechanism of resistance through studies of differential responses in the two strains to various kinds of implants, carcinogenic agents, infections and artificial lymphopenia.

HADORN, E., and NEEL, J., The University of Rochester, Rochester, N. Y.: *The accelerating effect of ring-gland injection upon puparium formation in normal and hybrid Drosophila larvae.*—Between the two hemispheres of the brain of *Drosophila* larvae, encircling the dorsal blood vessel, is found a ventrally incomplete ring of tissue, some  $200\mu$  in diameter. That this organ—the ring-gland—is the source of a hormone capable of accelerating puparium formation in *lgl/lgl* larvae has been shown by HADORN (1937). The present experiments indicate that this hormone has the same effect in  $+/+$  larvae. Injection of one to three genetically normal mature glands into genetically normal hosts by the EPHRUSSI-BEADLE method results in a significant acceleration of puparium formation. The degree of acceleration depends upon the time of the injection and the number of glands injected.—At  $25^{\circ}\text{C}$  normal flies have an egg-larval period of 4.5 days. The male larvae from the cross (*D. melanogaster* ♀  $\times$  *D. simulans* ♂) under very favorable cultural conditions form puparia at from 8.5 to 15.5 days after egg-laying; 50 percent have reached this stage within 11.5 days. When one normal ring is injected into such larvae at 8, 9, or 10 days subsequent to egg-laying, about 85 percent of the larvae produce puparia within 24 hours. Neither control nor injected male hybrids undergo further development subsequent to the initial form and color changes. It is suggested that one effect of hybridization is to throw the puparium-formation mechanism off balance—probably through a malfunctioning of the hormone-producing organ (the ring-gland) rather than through a deficiency on the part of the reacting material (larval skin).

HUETTNER, A. F., and SONNENBLICK, B. P., Washington Square College, New York, N. Y.: *Cytology of the egg of Drosophila melanogaster following X-radiation.*—In this, the first of a planned series of experiments, wild males were treated with 5000 r units and mated with untreated females. Eggs were collected and fixed at various periods of development. Upsets in the normal cytological picture were observed with marked frequency, the earliest cleavages being especially affected. The demonstrations will indicate several of these distorted figures.

HUMES, ARTHUR G., and SAWIN, PAUL B., Brown University, Providence, R. I.: *Homeotic variations in the axial skeleton of Mus musculus.*—An in toto examination by the Spalteholz method of five families of mice, including 221 individuals, indicates the typical number of cervical, thoracic and lumbar vertebrae to be 7, 13, and 6 respectively. Variations from this proportion are continuous but may be arbitrarily classified into seven types according to the presence or absence of an additional pair of either cervical or lumbar ribs or

both and also according to the vertebra to which the pelvis attaches. The distinct characterization of three families—Family N by entirely normal rib and vertebral number, C by a predominance of cervical ribs, L by lumbar ribs and Cl (40 generations inbred) by cervical ribs and bifurcated xiphisternae—suggests that the forces influencing this variation are genetic. Since in a cross between families C and L (family CL) both cervical and lumbar ribs predominate, additional rib units here appear to dominate their absence. Dominance however either is incomplete or influenced by modifying factors since the mean expressivity of extra ribs is less than in the parental families. Greatest expressivity and symmetry of lumbar ribs appears to be correlated with an increase in number of presacral vertebrae.

HUSKINS, C. L., HUNTER, A. W. S., NEWCOMBE, H. B., and WILSON, G. B., McGill University, Montreal, Canada: *Chromonema and chiasma studies in asynaptic, desynaptic and normal Trillium erectum*.—(1) Two types of asynapsis have been produced experimentally by abnormal temperatures in *Trillium erectum*. In one there is failure of pairing but close association between the pairs of sister chromatids. In the other there is lack of association between sister chromatids as well as between homologues. The direction of coiling and number of changes in direction have been determined in these and in normal plants.—(2) Desynapsis before metaphase following normal pairing and chiasma formation has also been produced experimentally and coiling intensity and changes determined in it. Conclusions from these two studies are: (a) correlation of direction in sister chromatids depends on closeness of association, (b) chiasmata cause a number of changes equal to the chiasma frequency, (c) the attachment is a point of random change and therefore causes half as many changes as there are chromatid attachments, (d) the remaining number of changes is proportional to the number of gyres. This varies greatly under the different conditions.—(3) It has been established that during diakinesis and metaphase when the chromosome is relatively stationary in size the chromonema is elongating. During the period of major spiral formation it at least doubles in length. Existing hypotheses of the mechanism of coiling are evidently invalid on these data; they suggest a new one.—(4) Individual chromonemata were traced through fifty pairs of chiasmata and 35 found to be of the compensating and 15 of the non-compensating type. A similar proportion was previously found in *Melanoplus*. There is a relationship between types of chiasmata and distance between them. These data are examined in relation to *Neurospora* and *Drosophila* genetic data. Chromatid interference definitely occurs in some organisms and may be of general occurrence, as its reported absence in *Drosophila* does not appear to be thoroughly established.

HUTT, F. B., Cornell University, Ithaca, N. Y.: *Naked, a sex-linked semi-lethal mutation in the domestic fowl*.—About three-quarters of the affected female chicks die during the last three days of incubation. Those hatched lack the normal covering but may have sparse strands of down. Shafts of the chick wing feathers may be extruded from the follicles but they are gnarled, twisted

and unable to develop normally. At maturity there is a fairly normal development of the fluff feathers in the abdominal and ventral regions but the contour feathers are markedly reduced in number and few of them have a normal web. The rectrices and remiges are absent or represented only by one or two very short shafts. In general appearance the birds resemble Silkie fowls but most have fewer feathers. Distribution and extent of the pterylae are normal. More than sixty affected chicks and embryos have thus far been obtained, all females. Chicks with normal down hatched from these matings show a 2:1 ratio of males to females, as is to be expected. The effect of homozygosity for the naked gene in the male is not yet known, but the character is obviously sex-linked, recessive and semi-lethal.

IRWIN, M. R., University of Wisconsin, Madison, Wis.: *A genetic analysis of species differences in Columbidae*.—Using standard immunological procedure, it can be readily demonstrated that, of two contrasted species of animals, each contains within its red blood cells antigenic substances "peculiar" to itself as well as other components "common" to both species. As a result of successive backcrosses to Ring dove, in the cross of Pearlneck (*Spilopelia chinensis*) by Ring dove (*Streptopelia risoria*) there have been isolated ten different cellular characters peculiar to Pearlneck. These individual components have been termed d-1, d-2, d-3, d-4, . . . d-10. Matings to Ring doves of backcross birds with these respective single characters produce offspring approximately half of which possess the particular Pearlneck character, and half do not. Such results would be expected on a genetic basis if the characters peculiar to Pearlneck were hereditary.—Three of these particular Pearlneck constituents have associated with them different parts of the new, or "hybrid" components, these being found in the cells of the species hybrid and not in those of the parental species. One or more parts of the "hybrid" components have been found in certain other species of doves.—These results represent experimental evidence showing that the differences in chemical pattern between species are of the same genetic order as the differences in pattern within species.

JONES, D. F., Connecticut Agricultural Experiment Station, New Haven, Conn.: *Variation resulting from unequal mitosis*.—Maize seeds heterozygous for the *C* aleurone color gene show colorless spots along with colorless spots paired with dark spots. The dark part of these twin spots may be uniformly colored or variegated. This variegation is secondary twinning, the dark cells reverting to normal or to colorless or changing to still darker. A few seeds show a high frequency of primary twin spots of regular outline. Other seeds show an extreme variation in aleurone color. This variable pattern is produced by colorless, normally colored and dark colored areas in irregular outline. These variegations occur in definite areas varying in size from small patches to the entire surface of the seed. In seeds heterozygous for the linked genes *C* and *Wx*, areas that have lost *C* are frequently variegated for *Wx*.



Similar behavior has been found for the *Pr* and *Su* genes. This type of variegation usually affects genes on only one chromosome but seeds variegated for both *C* and *Pr*, on different chromosomes, do occur. Variegated seeds are usually normal in growth but may show marked irregularity in cell coordination resulting in depressions and outgrowths. This type of variegation is clearly different from the common *R* variegation in seeds having one *R* allele and results from frequent losses or shifts of genes at successive cell divisions and is one of many manifestations of unequal mitosis during development.

KAUFMANN, B. P., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Complex chromosomal rearrangements following X-radiation of sperm of Drosophila melanogaster*.—Cytological analysis of complex chromosomal rearrangements in salivary glands of larval offspring of irradiated males has furnished information relative to 1) the organization of salivary chromosomes; 2) the structure of the chromosomes at the time of irradiation; 3) the mechanism of chromosome breakage and attachment.—1) Chromosomes which are broken close to the spindle fiber locus and attached to euchromatic regions reveal the extent of the heterochromatin belonging to the various limbs of the chromosomes, and confirm thereby the interpretation that the chromocenter is not an amorphous mass but a close approximation of heterochromatic portions of the component chromosomes. When proximal portions of the X and Y chromosomes are translocated to the limbs of autosomes, nucleoli frequently appear in those regions, indicating thereby the existence in salivary gland nuclei of specific nucleolus-organizing regions in the sex chromosomes. 2) Occurrence of duplicated sections of chromosomes suggests that the duplicated chromosome had at least two chromatids at the time of its irradiation in the sperm. In one complex rearrangement both chromatids of the duplicated section had been broken at two loci, but a third break occurred at different loci in each of the two strands. 3) This evidence suggests that breakage is not an effect of a direct electron hit since sister strands may be broken simultaneously. The most complex configurations analyzed, one revealing ten breaks and attachments of which five were in one chromosome limb, present serious difficulties for any simple explanation which regards overlapping of chromosomes as prerequisite to their breakage and rearrangement.

KIMBALL, R. F., Johns Hopkins University, Baltimore, Md.: *The determination and inheritance of sex at endomixis in Paramecium aurelia*.—In the sexually differentiated race S of *Paramecium aurelia* an individual of either sex, after going through endomixis may give rise to progeny, all of one sex, or all of the other sex, or of both sexes. In the latter case, all the progeny of one product of the first fission after the climax of endomixis are of one sex, and all the progeny of the other product are of the other sex. Since the segregation of sex at the first fission is correlated with the segregation of the macronuclei at this same fission, it is concluded that sex determination in this organism involves

the differentiation and segregation of the macronucleus. In the progeny of different sets of endomixis, two different but simple ratios of the two sexes are found; namely 1:1 and 1:2. In some clones the ratio changes from one of these to the other after the clone has gone through vegetative reproduction and endomixis. When the sex ratio is 1:1,  $\frac{1}{4}$  of the exendomixis give rise to progeny only of one sex;  $\frac{1}{4}$  to progeny of the other sex; and  $\frac{1}{2}$  to progeny of both sexes. When the sex ratio is 1:2, the ratio of these three classes is 1:4:4. Therefore the distribution of the two sexes to the two products of the first fission after the climax of endomixis is purely random.

LANDAUER, WALTER, Storrs Agricultural Experiment Station, Storrs, Conn.: *A lethal mutation in Japanese Bantam fowl*.—The "Standard of Perfection" requires that Japanese Bantam fowl shall have short lower thighs and very short shanks. It was found that individuals with short extremities, relative to the body, are heterozygous for a lethal mutation. Crosses (by artificial insemination) between Creeper hens and a Japanese Bantam cock have demonstrated that the lethal mutation is the same in the two breeds. The percentage of lethal embryos which develop to late stages is much greater in matings of Japanese Bantams than in Creeper matings. Even in the cross of Creeper  $\times$  Japanese Bantam about thirty percent of all homozygous embryos survived to late stages as compared with from three to eleven percent in Creeper matings.

LASHIN, BEATRICE J., New York University, New York, N. Y.: *Effect of temperature on the wings of dimorphos vestigial/pennant flies of Drosophila melanogaster*.—The curves of the mean wing length for the dimorphos vestigial/pennant males and females show an inverse response as the temperature increases from 16° to 28°, and are similar in slopes and values to those previously reported for wild type and homozygous not-dimorphos pennant. The male curve for mean wing area duplicates that of the homozygous not-dimorphos pennant males in slope and value between 16° and 28°. The curve for dimorphos vestigial/pennant female wing area is U-shaped and duplicates that of not-dimorphos vestigial/pennant females between 16° and 32°, but their wings are approximately double the area of those not-dimorphos vestigial/pennant. The male wings may be wild type but generally display minor nicks and notches. The female phenotypes are "antlered" and "strap" wing from 16° through 26°, with the highest frequency of "strap" at 22°. From 28° to 32° the wings are predominantly "nicked" or "notched." From both the length and area curves and the changes in phenotype, 28° appears to be a critical temperature for the dimorphos vestigial/pennant genotype.

LAWSON, CHESTER A., Wittenberg College, Springfield, O.: *Order and time of embryonic differentiation in relation to order of determination in gamic female aphids*.—The order of differentiation in gamic female aphids is (1) ovarioles (2) hind tibiae (3) colleterial glands and seminal receptacle. Ovarioles are dif-

ferentiated about the 7th day of embryonic development, the hind tibiae about the 10th day of embryonic development, the colleterial glands and seminal receptacle 2 or 3 days after birth (during 2nd instar). The order of determination is (1) ovarioles (2) colleterial glands and seminal receptacle (3) hind tibiae, which does not correspond to the order of differentiation. Gamic characters develop on the hind tibiae after the last molt (8 days after birth). If this event is considered to be the actual differentiation of the gamic hind tibiae the order of differentiation would be the same as the order of determination. On this assumption differentiation occurs at least 8 days after determination, as hind tibiae are determined before birth. Colleterial glands and seminal receptacle are likewise determined before birth, but do not appear until 2 or 3 days after birth, which supports the possibility of a delay in differentiation of hind tibiae. The time of differentiation varies, presumably as a consequence of some maternal influence.

LEBEDEFF, G. A., Cornell University, Ithaca, N. Y.: *The nature of intersexuality in Drosophila virilis*.—Genetical, morphological, cytological, and embryological (gonads only) studies of intersexes (LEBEDEFF, Proc. Nat. Aca. Sci. 1934) indicate that they start to develop as females. However, those sexual organs whose imaginal discs are laid down after the occurrence of the "turning point" develop into male organs. Thus the time of reversal determines the degree of intersexuality, as in the cases of *Lymantria* and *D. melanogaster* intersexes. Yet *D. virilis* intersexes differ morphologically from the others by being essentially hermaphrodites because of the peculiarity in the development of those organs whose imaginal discs had not become fully differentiated at the time of reversal. Such organs, with the exception of a few secondary ones which are transformed into corresponding male organs, continue to develop as female organs. But along with them, male organs appear, presumably from fresh outpushings from the imaginal discs. The two systems, the original female and the additional male, develop side by side resulting in hermaphrodites. The development of gonads is somewhat different. After the occurrence of the "turning point," female germinal cells in the ovaries of intersexes are gradually transformed into male-like ones. The ovary itself is not transformed into a testis, except in very early reversals, but it buds out a testis-like organ, to which migrate some of the oocytes now transformed into spermatocytes. The two organs are attached to each other.

LINDSTROM, E. W., Iowa State College, Ames, Ia.: *Maternal influence in the heredity of tillering in maize*.—In certain reciprocal, single crosses between tillered and single-stalked inbred lines of dent corn, a significant maternal influence on tillering was evident in each of three years. The higher percentage of tillering (10, 14 and 26 percent in the three years) occurred when the female parent of the crosses was the tillered strain. Reciprocal backcrosses of such F<sub>1</sub> plants to both the single-stalked and the tillered parental lines gave differences of 12 and 18 percent in the same direction. In these backcrosses, degrees

of tillering within the tillered class also gave evidence of the greater maternal effect. In the reciprocal backcrosses, any direct effect of seed or embryo size on tillering was ruled out by the fact that in one backcross the tillered mother plants ( $F_1$ ) had the larger seeds and embryos, whereas in the other reciprocal backcross the tillered female was the parental inbred strain with smaller seeds and embryos. Apparently tillering primordia of the embryo develop under some influence of the mother plant. The inheritance of tillering is multigenic in nature, complicated not only by climatic and nutritional agencies, but also by this maternal effect.

LOEWING, W. F., University of Iowa, Iowa City, Ia.: *The photoperiod as a factor in sex organ inception in flowers*.—When stem apices of certain short day plants such as hemp are exposed to an 8 hour photoperiod, inception of stamens is initiated but not of pistils. When terminal leaves are given short photoperiods, pistillate flowers are initiated both in high and low light intensity even when the latter is below the photosynthetic threshold. Defoliation and complete darkening of leaves, but with stems illuminated, produce similar responses on pistil formation, suggesting that the leaf is the perceptive locus rather than primarily the seat of requisite food for the response.—Metabolic changes in the staminate loci precede and are different from those occurring in the regions of pistil formation under short day conditions. Respiration, sugars, oxidase, phosphatase and diastase action are higher in stamen loci while pistil loci are marked by an increase in reducase, and amino-N. High nitrogen cultures of hemp produce only females, and nitrogen-deficient cultures only males. These responses suggest that loci high carbohydrate (and low nitrogen) favor stamen and high nitrogen pistil formation in flowers. The nutritional readjustments follow perception of short photoperiods by different organs of the plant.

LOVE, R. MERTON, Dominion Experimental Farms System, Ottawa, Canada: *A cytogenetic study of white chaff off-types occurring spontaneously in Dawson's Golden Chaff winter wheat*.—Spikes of normal, heterozygous (indistinguishable from normal), and homozygous white chaff plants are shown, as well as photomicrographs of meiotic chromosome complements of the three genotypes. Normal Dawson's has 21 bivalents, all chromosomes having median or sub-median centromeres. The heterozygote gives a monofactorial ratio and is characterized by a heteromorphic bivalent, one chromosome of which has a terminal centromere due to the loss of the shorter arm. White chaff plants are homozygous for the deficiency. Genes for glume length are linked with those for glume colour since the white chaff plants have longer glumes than the normal.—Evidence from another line which included a 41-chromosome plant with a duplication on the univalent indicates how such a deficiency can arise. Pairing of the duplicated segments of the univalent resulted in chromosome breaks at first anaphase, usually in the region of the centromere. This plant gave rise to a 42-chromosome plant with a heteromorphic bivalent similar to that described above.

MACARTHUR, JOHN W., University of Toronto, Toronto, Canada.: *Genetics of multiple births*.—Twins, triplets, quadruplets and quintuplets offer favorable material for a comparative study. The trends through the multiple birth series with regard to the sex distribution in the sets, the age and parity of the mothers, and the incidence of further multiple births throw some light on several genetic problems, particularly the nature of the inheritance of the proclivity to produce multiple births.

MACKNIGHT, R. H., California Institute of Technology, Pasadena, Cal.: *Cytology of Drosophila miranda*.—There is present in the salivary gland nucleus of the male of *Drosophila miranda* a chromosomal element consisting of a number of short euchromatic segments, most of which are attached at both ends to the chromocenter. Since this element occurs in males but not in females, it represents the Y chromosome or a part thereof. Most of its euchromatic material is thought to be homologous to the third chromosome of *Drosophila pseudoobscura*.

MARSHAK, ALFRED, New England Deaconess Hospital, Boston, Mass.: *Alteration of sensitivity of chromosomes to X-rays*.—Chromosomes in mitosis are most sensitive to X-rays at the onset of the prophase when their chromonemata divide. The time interval from this stage of maximum sensitivity to anaphase is the same (2.5–3.0 hours) for all organisms studied. The sensitivity of chromosomes may be expressed by the equation  $Y = e^{-k}$ , where  $Y$  is the percent normal anaphases and  $k$  the slope of the survival curve.  $k$  varies with the species used, its magnitude being directly proportional to the lengths  $l$  of the chromonemata. This relationship holds for all the tissues which have been investigated thus far: the mouse sarcoma CR180, rat carcinoma Walker 256, root tips of the broad bean, pea, onion, and tomato, which represent a wide range of values of  $l$ . From  $l$  and  $k$  the diameter  $d$  of the X-ray sensitive portion of the chromonema may be calculated. In the three organisms where these have been determined the value of  $d$  is  $10\text{--}20 \times 10^{-8}$  cm. This would correspond with the average separation of polypeptide chains as obtained from X-ray diffraction studies of proteins, or with the approximate short diameter of a protamine or histone. One would expect from this value and from work on the precipitation of proteins by X-rays that the reactive chromosomal material carries a net positive charge. To test this hypothesis further seedlings of two species treated with different concentrations of  $\text{CO}_2$  and  $\text{NH}_4\text{OH}$  were irradiated and the frequency of chromosome abnormalities determined. The results show that  $\text{CO}_2$  has no effect while  $\text{NH}_4\text{OH}$  inhibits the chromosome-breaking action of the X-rays, which is taken as evidence in support of the hypothesis. After treatment with a solution of .008 N  $\text{NH}_4\text{OH}$  the X-ray effects differ from those of the controls by a factor of more than three. This difference decreases with decreasing concentrations of ammonia.

MCCLEINTOCK, BARBARA, University of Missouri, Columbia, Mo.: *A method for detecting potential mutations of a specific chromosomal region.*—Plants having two deficient chromosomes-5 (the deficiency included the locus of  $Bm_1$ , allele of  $bm_1$ , brown mid-rib, producing a brown color in the lignified cell walls) and a small ring-shaped fragment covering the deficiency and carrying  $Bm_1$  are mosaics of heterozygous and homozygous deficient tissues through frequent losses of the ring fragment during somatic mitoses. This homozygous deficient tissue is (1) much reduced in growth capacity, (2) contains no chloroplasts, (3) has the characteristics of  $bm_1$  in its cell walls and (4) dries on exposure to sunlight. Relatively infrequently, during somatic mitoses, the ring chromosome increases or decreases in size through duplications or deficiencies of segments of chromatin composing the ring. Duplicated segments produce no obvious tissue modifications. If removal of specific regions from the ring results in homozygous deficient tissues having specific modifications, several types of mutant sectorials, depending upon the region removed, should be repeatedly encountered in large populations of such plants. The following types of "simple" mutant sectorials have been found: (1) transparent white with colorless cell walls, no plastids; (2) opaque white with colorless plastids, colorless cell walls; (3) deficiency- $bm$ , similar in detail to normal  $bm$ ; (4) pink colored tissue with colorless cell walls, colorless plastids; (5) blotched chlorophyll pattern, colorless cell walls. The following types of "compound" mutant sectorials have been found: (1) pink, deficiency- $bm$ , viable in sunlight; (2) pink, deficiency- $bm$ , dries in sunlight; (3) opaque white, deficiency- $bm$ ; (4) blotch, deficiency- $bm$ , dries in sunlight; (5) blotch, dries in sunlight. On the theory that compound mutant sectorials are the product of losses of several adjacent regions of chromatin, the simple mutant effects are referred to the chromosome in the following order: pink, deficiency- $bm$ , dries in sunlight and blotch, with translucent white removed from deficiency- $bm$  and opaque white close to it. Since a homozygous deficiency for the  $Bm_1$  locus produces the same effect as the known gene  $bm_1$ , it is possible that these other mutants may eventually appear as "genes" closely linked to  $bm_1$ .

METZ, C. W., Carnegie Institution of Washington, Baltimore, Md.: *Structure of the "puffed" regions in giant salivary gland chromosomes in Sciara.*—In the giant chromosomes of *Sciara ocellaris* Comst. certain particular regions sometimes appear greatly expanded or "puffed." These possess several features of special interest. When not "puffed" they exhibit the characteristic banded structure of other regions. When greatly "puffed" they appear almost uniformly granular with a structure resembling that of the cytoplasm (in fixed preparations). Intermediate degrees of "puffing" show the bands or discs in various stages of disruption. In the puffed condition a region is apparently as long as in the non-puffed condition, indicating that it contains much more material. The extra material is apparently all achromatic, in the form of small droplets. The chromatin is apparently not increased in amount, but there appear to be many more granules. No evidence of chromonemata has

been detected.—The material in these puffed regions may be comparable to that of the "chromocenter" in *Drosophila*. If so, the present evidence supports the view of SCHULTZ, BAUER and others that the chromocenter is not inert, and also the conclusion of PROKOFYEVA-BELGOVSKAYA that its fundamental organization is like that of other regions of the chromosomes.—Studies on these chromosomes also raise a question as to the significance of the distinction frequently made between "heterochromatin" and "euchromatin." In *Sciara* there appear to be many structures intermediate between those typical of these two categories, and some structures are apparently capable of changing from the one condition into the other.

MICKEY, GEORGE H., University of Texas, Austin, Tex.: *Effect of temperature on frequency of translocations produced by X-rays*.—A reduction of temperature to 4°C during X-radiation of *Drosophila melanogaster* produced an increase in the rate of translocations to almost double the percentage obtained by irradiation at room temperature. Using the frequency of translocations between the second and third chromosomes as a criterion, the effectiveness of X-radiation at different temperatures was tested by treating two groups of wild type males simultaneously (thereby absolutely controlling the dosage): the one at room temperature, which varied from 28°C to 33°C; the other at a temperature of  $4 \pm 1^\circ\text{C}$ . Thus the minimum difference amounted to 23°C and the maximum, 30°C. One experiment yielded 4.91 percent translocations of the second to third chromosomes out of 1221 F<sub>2</sub> cultures whose P<sub>1</sub> males were X-rayed at 4°C, and 2.57 percent among 1553 F<sub>2</sub> cultures from P<sub>1</sub> males irradiated at the higher temperature. A rate of 4.32 percent translocations was obtained at 4°C in a second experiment involving 1636 F<sub>2</sub> cultures; while 2.6 percent was produced at room temperature out of 1693 F<sub>2</sub> cultures. A similar acceleration of rate was exhibited in the translocations between the X chromosome and second, and between the X and third chromosomes.

MULLER, H. J., and RAFFEL, DANIEL, Academy of Sciences of U.S.S.R., Moscow, U.S.S.R.: *The manifestation of the position effect in three inversions at the scute locus*.—MULLER and PROKOFYEVA (1935), demonstrated that the "mutations" at the scute locus produced by X-radiation are often due to the altered position of the genes in the chromosome and not to changes in the scute gene and showed that the inversions associated with *sc*<sup>4</sup>, *sc*<sup>L8</sup>, and *sc*<sup>SI</sup> have their breaks at the same points or at least that there are no genes necessary for the life of the flies between the breaks of these inversions. The effects of these inversions and combinations of the ends of the different ones with each other in stocks isogenic for all II and III chromosome genes and for all X chromosome genes between *w* and *car* are shown in the demonstration. The differences found between them are due either to a gene not otherwise demonstrable which may lie between the left break of *sc*<sup>4</sup> and the other two breaks or else to the differences in the right breaks that have already been demonstrated.

MULLER, H. J., PROKOFYEVA-BELGOVSKAYA, A. A., and RAFFEL, D., Institute of Genetics of the Academy of Sciences of the U.S.S.R., Moscow, U.S.S.R.: *The absence of transmissible chromosome fragments resulting from simple breakage, and their simulation as a result of compound breakage involving chromocentral regions.*—Fourteen scute changes produced by irradiation of structurally normal X chromosomes included no losses of entire distal end. Considering the chromosome fragmentation reported by cytologists following irradiation, this indicates inconvertibility of ordinary interstitial into terminal genes by chromosome breakage. However, LEVIT's irradiation experiments (1931) on scute-8 apparently produced many distal-end losses by breakage of the interstitial chromocentral region adjoining achaete. The same phenomenon occurs spontaneously comparatively often. Salivary study shows these "broken" scute-8 chromosomes still contain the distal end in situ, though often giving evidence of minute rearrangement near achaete. Thus chromocentral and adjoining regions are especially predisposed not merely to breakage (without restitution) but also to compound breakage with minute rearrangement (deletion, inversion). Similarly, gross deletions and inversions of normal X chromosomes, having two distant breaks including one in proximal chromocentral region, commonly contain an additional chromocentral break nearby, with resultant rearrangement of bobbed with respect to block-A. It seems probable that the few reported single-break deficiencies, inversions, and translocations of normal chromosomes involve second breaks in the terminal chromocentral regions recently found by the second author.

NABOURS, ROBERT K., and MACQUEEN, NELLE R., Kan. Agric. Expt. Sta., Manhattan, Kan.: *Wing and pronotum length in grouse locusts.*—Dimorphism in wing length, with corresponding length of pronotum, is common among the grouse locusts (Sub-fam. Tetriginæ). In several species (*Paratettix texanus*, *Apotettix eurycephalus*, et al.) the offspring which mature in seasons favoring rapid growth (spring and early summer) show marked preponderance of the long-winged phase; while short-winged individuals predominate in fall and winter, when growth is slow. These phases are not dependent upon the wing length of the parents. (Previously published.)—Although the same apparent dimorphism exists in the species *Tettigidea lateralis* Scud., the long-winged is recessive, and breeds true consistently, regardless of the season or other features of the environment. Heterozygotes bred with recessives produced 1238 short-winged to 1116 long-winged. Heterozygous pairings, however, gave 548 short-winged to 259 long-winged, approximately a 2:1 ratio.—These ratios indicate (1) that a lethal may be linked with short-winged; and (2) that dimorphism in *T. lateralis* is due to one or more factors quite different from those operating in several other species of the Tetriginæ.

NEBEL, B. R., and RUTTLE, M. L., New York State Agricultural Experiment Station, Geneva, N. Y.: *Action of colchicine on mitosis.*—The action of the alkaloid colchicine on mitosis was studied in the following material: stamen



hairs of Tradescantia, roots and shoots of Pea, Tomato, Tagetes, Antirrhinum, Trifolium, Papaver, Dianthus, Solanum and Lilium—testes of *Podisma*, eggs of *Asterias* and *Arbacia*. The active concentrations of colchicine are, in all tissues investigated, similar if not identical:  $5 \times 10^{-4}$  to  $5 \times 10^{-5}$  will stop mitosis during metaphase. In concentrations  $5 \times 10^{-5}$  to  $10^{-6}$  binucleate cells may be produced and divisions may be irregular. Colchicine is a more specific chromatin poison than phenyl-amyl-propyl, ethyl methane or chloral hydrate. With the *Arbacia* egg it was found that with colchicine  $10^{-5} \times 5$  cleavage and astral rays no longer appear. The spindle is reduced in size and chromosomes are slowed in metaphase. The rhythm of nuclear division may persist so that when controls are in third cleavage metaphase, eggs to which colchicine was added 10 minutes after fertilization will also show approximately 4 groups of chromatin, but the plates are hypoploid often containing only 1 to 6 separate chromosomes ( $2n=36$ ) which may represent fused units. The micronuclei which form are not far apart from one another and their resting stage is relatively short. Colchicine deserves further study as an agent which may induce polyploidy and mutations. It may be used as a tool in chromosome counting and may prove useful in studying secondary pairing and other interchromosomal relationships.

OLIVER, C. P., University of Minnesota, Minneapolis, Minn.: *A chromosomal unbalance in Drosophila melanogaster which imitates the gene facet*.—In a translocation marked with Dichaete, an inner fragment of III (*sr-e*) is inserted near *ec* in X. Males with a duplication of the fragment have many characteristics of facet, and with Notch behave like facet-Notch.—Duplication males have eyes roughish, between *fa* and +, wings slightly spread and occasionally nicked, heavy body pigment, weak crossveins and coarse, occasionally doubled bristles. Duplication females usually lack the rough eyes and nicked wings. Heterozygous Notch-duplication females have eyes smoother than Notch-facet, but wings more widely spread, more extremely notched, narrower and shorter.—Crossing over is decreased fully sixty percent in the *sc-ec* and *ec-ct* regions. In earlier crossover counts with Tx-3D/old-xple MC3x, three *ec ct D* occurred among 1209 offspring, indicating that the insertion is left of *ec*. All other types fit this interpretation. Among 444, using T(2-3, Cy C3x), one *ec ct D* and one *ct v g Cy* appeared. The latter will not fit the interpretation, unless it is a duplication. All others do fit. In recent tests (1694 total) using Xple without C3x, two *sc ec D* males appeared; both died without offspring. Either they were third chromosome crossovers, or they indicate that the insertion may be right of *ec*; and cytologically the insertion apparently is between 4F<sub>2</sub> and 4F<sub>3</sub> (GRIFFIN).—The duplication of III may cause the morphological changes. Preferably, with the insertion something else occurred in the region near *fa*. This may have been a deletion so that males with the duplication of III lack something of *fa*.

PATTERSON, J. T., STONE, W. S., and SUCHE, META, University of Texas, Austin, Tex.: *Aneuploidy of the second and third chromosomes of Drosophila*

*melanogaster*.—By means of nine II–IV, and nineteen III–IV translocations, hyperploids were obtained for nine regions in chromosome II and thirteen in III, covering the entire chromosome in both cases with the exception of one untested section in each chromosome, and an additional region in IIIR for which no aneuploids were recovered. Additional hyperploids were obtained for five shorter and one longer section in III. Hypoploids were obtained for one region in II and twelve regions in III, the sections in III being relatively shorter. The frequency of recovered aneuploids varied. Hyperploids were usually more frequent than hypoploids for the same region. Phenotypically both types of aneuploids were in many cases nearly normal. Hyperploid variations included wings slightly to widely spread, curved downward, or raised; enlarged wing cells; eyes rough, body shorter, broader. Hypoploids in some cases showed greater variation from normal in coarser wing texture, darker body color, disarranged facets, fewer bristles, or shortened legs and antennae. Genitalia were usually normal, rarely rotated. Tests of aneuploids showed a wide range of viability and fertility, at times equal to normal, usually less. Hypoploids were generally less viable and fertile than hyperploids. With two exceptions males proved more viable and fertile than the corresponding aneuploid females. The greater tolerance of autosomal aneuploidy by males as compared to females is the reverse of the X chromosome condition.

PLOUGH, H. H., and CHILD, G. P., Amherst College, Amherst, Mass.: *Autosomal lethal mutation frequencies in Drosophila melanogaster*.—By an extension of the Cy/Pm and SbC/2C Dfd method for detecting lethals in the second and third chromosomes of *D. melanogaster*, the frequency of lethal mutations per generation was obtained. The determinations were made at constant temperature for a number of successive generations, and after a number of generations during which an accumulation of lethals was allowed to occur. The results show that there is an appreciable rate of mutation under constant environmental conditions, the rate being slightly higher in the third than in the second chromosome.

RHOADES, M. M., U. S. Department of Agriculture, Washington, D. C.: *On the origin of a secondary trisome through the Doubling of a half-chromosome fragment*.—Maize plants hyperploid for a chromosome fragment, consisting of the entire short arm of chromosome V and possessing a terminal spindle fiber attachment region, occasionally produce an aberrant type. These offtype plants are secondary trisomes in which the extra chromosome consists of two short arms of chromosome V so joined as to have a median attachment region. Genetic data show that the fragment chromosome is involved in the formation of these secondary trisomes. When plants hyperploid for the fragment were used as the male parent there were 47 secondary trisomes among 11,293 individuals (0.42 percent). Fragment plants used as the egg parent gave 7 secondary trisomes in 3253 plants (0.22 percent). The percentage of the offspring hyperploid for the fragment chromosome is 30.3 when hyperploid plants are

used as the female and only 0.40 percent when used as the male. This difference results from the inability of pollen hyperploid for the fragment to compete successfully with haploid pollen while there is no selection on the female side. However, the percentage of secondary trisomes is as great, at least, through the pollen as through the eggs. But the functional pollen from secondary trisomes consists entirely of haploid grains indicating that pollen hyperploid for the "secondary" chromosome rarely, if ever, functions. As an explanation of the occurrence of secondaries through the pollen it is suggested that occasionally in the development of those gametophytes hyperploid for the half-chromosome fragment, the "secondary" chromosome is produced by a doubling of the half-chromosome fragment. Reduction takes place in the succeeding anaphase. One pole receives no fragment chromosome while the other receives both fragments which have become incorporated into a single chromosome with a median attachment region. If, in the first post-meiotic division of the male gametophyte, the tube nucleus becomes haploid by reduction following the formation of the "secondary" chromosome, the growth of the pollen tube presumably would be normal but the sperm would carry the secondary chromosome.

RILEY, HERBERT PARKES, Newcomb College, Tulane University, New Orleans, La.: *Interlocked bivalents as a cause of polyploid pollen grains*.—A plant morphologically like *Tradescantia paludosa* was found in a colony of plants which were chiefly tetraploid hybrids showing characters of any two or three of the species, *T. paludosa*, *T. canaliculata* and *T. hirsutiflora*. This plant was a diploid but produced a small percent of  $2n$  pollen grains. Almost all the cells in the metaphase of the first meiotic division had six pairs of chromosomes, but about two percent had four pairs and a ring of four. Twenty-seven percent of the cells had interlocked bivalents. The chiasma frequency was 2.00 per bivalent for cells with interlocked bivalents and 1.97 for the other cells. No univalents were found in any cells. At anaphase, over twelve percent of the cells showed a 5-7 distribution of the chromosomes and about six percent showed lagging or "fused" chromatids similar to those which have been produced by temperature changes. At the microspore division, from ten to fifteen percent of the cells in metaphase or anaphase had twelve chromosomes instead of the normal six; over eight percent of cells in all stages of the microspore division were roughly twice the normal size. Pollen fertility was high (about 95 percent) and about ten percent of the mature pollen grains were giants. Apparently the plant is a remote hybrid and the interlocked bivalents are probably due to a small segmental interchange. Some of the interlocked bivalents separate producing no further abnormality, but some fail to pull apart and produce non-disjunction or lagging chromosomes. The lagging suppresses cytokinesis and this results in tetraploid pollen grains.

RUSSELL, ELIZABETH SHULL, Jackson Memorial Laboratory, Bar Harbor, Me.: *A quantitative study of genic effects on guinea-pig coat color*.—In order to measure dominance relations in the coat color factors of the guinea-pig, and

the effects of each gene replacement in different genetic backgrounds, determinations have been made of the amount of pigment in the hair of various genotypes. Colorimetric comparisons of alkaline solutions of the pigment were used to determine the relative concentrations in various yellow types. In sepias, the pigment was isolated by the EINSELE technique and its amount determined by weighing or oxidizing with potassium permanganate. Studies have been made of the effects of the albino series,  $C$ ,  $c^k$ ,  $c^d$ ,  $c^r$ ,  $c^a$ , affecting the intensity of all colors;  $E$ ,  $e$ , determining the difference between sepias and yellows;  $P$ ,  $p$ , determining the difference between intense and pale sepias; and  $B$ ,  $b$ , determining the difference between black and brown. The results for the albino series of alleles indicate that in sepias, pale sepias, and yellows, the homozygotes of the lower active alleles produce approximately twice as much pigment as their heterozygotes with the inactive gene or genes, while the highest allele appears to be completely dominant. This suggests that in some reaction between gene and character the gene product is in defect in relation to the substrate with the lower alleles and in excess with the highest member of the albino series.

SATINA, S., BLAKESLEE, A. F., and AVERY, A. G., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Chromosome behavior in triploid Datura stramonium. III. The seed.*—Female gametes contain nuclei with all possible numbers of chromosomes between  $1n$  and  $2n$ . Each ovary has several hundred ovules but in  $3n \times 2n$  crosses less than one quarter become fertilized. Fertilization, however, takes place regardless of the number of chromosomes in the gametes, although no offspring with more than 27 chromosomes have been identified in *Datura*. The size of the fertilized ovules and the speed of development vary considerably. A large number of fertilized ovules disintegrate soon after fertilization or later; others stop development at rather advanced stages. This explains the small number of good seeds and the presence of defective seeds in capsules. One thousand seeds from 15 capsules were measured and seed-coats removed. The size varied from 1.7–4.9 mm; 388 seeds were good, 120 defective and 492 consisted of seed coat only. Character of seeds was unrelated to size. From the 436 seeds sown, 399 germinated within 3 to 163 days, 114 seedlings and older plants died chiefly because of poor viability. The remaining 285 offspring consisted of 58— $2n$ , 137— $2n+1$ , 80— $2n+1+1$  and 10— $2n+1+1+1$  plants. A relation exists between the size of seeds and the chromosome which is extra:  $2n+1 \cdot 2$ ,  $2n+13 \cdot 14$  and  $2n+15 \cdot 16$  plants develop from small seeds;  $2n+23 \cdot 24$  plants from large seeds. Forty-six seeds had delayed germination of more than 100 days. In all these cases (except 4) the 3·4, 13·14 or 15·16 chromosomes was present as an extra. Apparently the 1·2 and 21·22 chromosomes speed up germination in combinations with other extra chromosomes.

SAWIN, P. B., WHEELER, K. M., STUART, C. A., and GRIFFIN, A. M., Brown University, Providence, R. I.: *Inheritance of normal agglutinins for human erythrocytes in rabbit serums.*—Serums of domestic rabbits may be classified

according to their natural ability or inability to agglutinate human erythrocytes of groups O, A and B. Many serums possess antibodies for more than one cell type and the specificity of each may be determined by adsorption tests. The presence of normal specific alpha agglutinins (for A cells) is inherited as a Mendelian recessive. All of the 77 animals in family I possessed these agglutinins. Among 350 individuals of seven other unrelated families only 131 or 37.4 percent possessed the antibody. From matings within these seven families, typical  $F_2$  and backcross ratios of negative (lacking alpha agglutinins) to alpha were obtained. Crosses between heterozygous males of negative families and family I produced 19  $F_1$ , 12 of which had alpha agglutinins. A similar disproportion occurs in the backcross of the negative  $F_1$  to family I rabbits, suggesting a possible genetic duplication of the alpha gene in certain of these families. Extracted alpha parents breed true. The age at which normal alpha agglutinins are actively produced varies from 2 to 4 months. Tests of newborn young of the several possible genetic derivations show that with few exceptions, alpha agglutinins are absent at birth in the progeny of negative mothers, but occur in 50 or 100 percent of the young from alpha mothers according to the heterozygosity or homozygosity of the father for the alpha character. The ability to produce high titrating immune alpha agglutinins depends on the presence of the normal alpha agglutinin.

SHRIGLEY, E. W., University of Wisconsin, Madison, Wis.: *Qualitative and quantitative differences in the morphology of spermatozoa from members of two dove species, and from their  $F_1$  and backcross hybrids.*—Ring doves and Pearlnecks as well as their  $F_1$  and backcross hybrids have been examined for the presence of normal and abnormal spermatozoa. Observations were made on "living" and fixed material from the vasa deferentia, and on fixed smears and paraffin sections of the testes. It was possible to classify the morphological variations of the heads of the gametes into the following six groups: (a) normal appearing sperm, (b) spermatozoa whose heads were associated with varying amounts of cytoplasm, (c) cells possessing branched heads, (d) giant-headed sperm, (e) atypically stained heads, and (f) heads of gametes possessing bulbous swellings. Attempts failed to produce anomalies in the sperm head comparable to any described, with the exception of the atypical staining reaction. There was an indication that the bulbous and branching abnormalities became manifest in the early stages of spermiogenesis.—Each of the four bird populations was found to possess all six classes of sperm and to vary with respect to one another in the quantity of these classes. Variations in the percentages of normal and abnormal sperm among the various bird groups suggested a possible genetic influence. The  $F_1$  hybrids possessed the highest proportion of abnormalities, and the backcross hybrids, as a group, resembled the parent species, but showed extensive individual variability. Further, there was an indication among the backcross birds that the proportion of sperm abnormalities was associated with the degree of serological relationship to the  $F_1$  hybrids.

SHULL, A. FRANKLIN, University of Michigan, Ann Arbor, Mich.: *Time of determination and time of differentiation of aphid wings*.—The time of determination of wings in aphids has been known. Since the early stages of wing development consist of thickenings of the hypodermis in four definite areas, the time of differentiation can be rather accurately decided. Whether an aphid is to be winged or wingless can be known almost with certainty from the treatment (light and temperature) it has received. Embryos shortly before birth, and the first instar, have the wing rudiments in characteristic position even if they are destined to be wingless; but they are thinner and grow less laterally than do those of winged individuals. The additional thickening and the tangential growth which marks future wings starts shortly before birth. The first signs of such development are found in embryos which have already started to form red pigment in the eyes. From the number of red-eyed embryos in a female, and the birth rate of her offspring, it is concluded that pigment formation starts about 26 hours before birth, on the average. Wing buds therefore start their definitive thickening perhaps 22 to 24 hours before birth. This is only a few hours after the determination of wings, as ascertained from earlier experiments.

SMITH, E. LUCILE, and ROBERTSON, W. R. B., State University of Iowa, Iowa City, Ia.: *A new mutant eye color in Drosophila melanogaster*.—From one of the  $F_1$  inter se matings of the controls in an X-ray experiment being carried out on a wild type stock secured from MORGAN, two offspring had dark eyes and fourteen had normal eyes. The character bred true. The eye color changes from pinkish buff in the one-day-old to dark red brown in the week-old fly, at which time it is distinctly darker than *bw*. It has, provisionally, been called brown-b. In matings of Star/Curly with brown-b  $F_1$  Star males or females to brown-b females or males indicated independent assortment. The same was found in crosses of brown-b with Bristle vestigial-Depilate. In matings of brown-b with Lyra/Dichaete both Lyra and Dichaete  $F_1$  males gave no recombinations when backcrossed to brown-b, but  $F_1$  Lyra and Dichaete females gave among 11,298 offspring 5030 crossovers, 44.52 percent, which (with the correction from recombination curve) located brown-b on the 3rd chromosome at 97.0. In crosses with Stubble/ $C_3l_3a$   $F_1$  females gave among 9255 flies 3643 crossovers, or 39.36 percent, which with the correction located brown-b at 100.36. In a similar cross with Hairless Minute-w/ $C_3el$   $F_1$  Hairless Minute-w females by brown-b males gave among 9555 offspring 2314 (24.22 percent) crossovers between Minute-w and brown-b and 2975 (31.14 percent and counting visible double crossovers twice) crossovers between Hairless and brown-b; thus locating brown-b at 104.22 with reference to Minute-w and at 101.34 with reference to Hairless. Further matings are needed for accurate location. In crosses of brown-b to brown (*bw*)  $F_1$  have wild-type eyes. In  $F_2$  the double recessive has permanently very light buff eyes.

SMITH, LUTHER, University of Missouri, Columbia, Mo.: *Cytogenetic studies in Triticum monococcum*.—(A) Genetic factors for sterility. Nineteen cases of sterility resulting from X-ray treatments have been found, eleven of which are associated with abnormalities of the meiotic process in PMC. These eleven, which apparently are inherited as simple recessives, belong to the following five types: (a) No bivalents are present at diakinesis and IM. (b) A variable number of bivalents are present at diakinesis and IM. (c) The chromosomes break up into numerous fragments at IA, but macrosporogenesis is presumably normal, since these plants produce many seeds when pollinated with normal pollen. (d) Diakinesis and IM chromosomes are shorter than normal, and univalents are commonly present. (e) Frequent dyad formation occurs, resulting in diploid microspores and numerous tetraploid offspring. The other eight cases include three in which the anthers abort, before or shortly after meiosis; four in which meiosis is normal but the mature pollen is devoid of starch; and one in which the contents of the microspores are aberrant.—(B) Meiotic rings. Plants with rings of four chromosomes have about 6 percent; those with rings of six about 27 percent; and two rings of four about 22 percent reduction in seed set. Pollen abortion in these three types is correspondingly low, although segregation of the chromosomes in PMC appears not to be directed.—(C) Chromosomal variants. Three types have been studied: (a) Haploids. (b) Triploids. (c) Trisomics, obtained from plants with irregular meiotic behavior or with a ring of six.—(D) Linkage studies. Five linkages involving 10 genes for seedling and mature plant characters have been found.

SONNEBORN, T. M., Johns Hopkins University, Baltimore, Md.: *Sex behavior, sex determination, and the inheritance of sex in fission and conjugation in Paramecium aurelia*.—As already reported for race S of *Paramecium aurelia*, the individuals of another race, R, are also sexually differentiated. In both races, all the descendants of a single individual (in the absence of endomixis and conjugation) are of the same sex as the progenitor. When cultures of different sex are brought together, the animals unite at once for conjugation in agglutination-like clusters; this will be shown. In both races sex is inherited at conjugation in simple ratios: either 1:1 or 1:2. When the ratio is 1:1,  $\frac{1}{4}$  of the exconjugants produce descendants of one sex only,  $\frac{1}{4}$  produce the other sex only, and  $\frac{1}{2}$  give rise at the first fission to one individual with progeny of one sex only and one individual with progeny of the other sex only. When the sex ratio is 1:2, these three classes of exconjugants appear in the ratio 1:4:4. Sex of the individual is not determined by the micronucleus as amiconucleate races are also sexually differentiated. That sex is determined by the macronucleus is shown (a) by the segregation of sex at the first fission after conjugation, when the two new independently formed macronuclei are segregated; and (b) by its occasional segregation at the second fission in race R, in which 3 or 4 instead of 2 macronuclei are sometimes formed in the exconjugant. In race R, there arise occasionally lines in which the individuals can mate among themselves, though they are genetically of the same sex, as shown by tests on

split pairs. The sex ratios from such matings are the same as in matings between the two sexes, showing that the sex factors segregate at the third maturation division.

SONNENBLICK, B. P., New York University, New York, N. Y.: *X-radiation of the mature gametes of Drosophila melanogaster: Cytologic abnormalities and egg mortality*.—When adult flies of either sex of a vigorous Oregon-R race are irradiated and then mated with untreated virgins, a high percentage of the deposited eggs do not hatch as larvae. Dosages ranging from 2300 r units to 5000 r units were employed and counts of the zygote mortality taken at each dosage. More than five hundred cytological preparations of these eggs in stages from first cleavage to shortly before hatching have been prepared, as have a set of control preparations.—Distorted mitotic divisions with chromosomes and chromosome fragments lying free in the cytoplasm have been found, as have frequent polyastral configurations with the chromosomes advancing to the three or four poles as the case may be. Vacuolization of the cytoplasm and swollen nuclei have been seen. Clumping of the chromosomes is a characteristic phenomenon; a mechanism to account for the clumping is offered. A number of eggs have gone on to form syncytial, undifferentiated masses, whereas control eggs of similar age have become highly differentiated. Thus, nuclei containing treated chromatin may divide actively, but the eggs containing them occasionally will not become differentiated.—It is probable that there is a direct relationship between the cytologic abnormalities mentioned and the high percentage of egg mortality due to X-radiation of the mature gametes.

SONNENBLICK, B. P., and HUETTNER, A. F., Washington Square College, New York, N. Y.: *Cytology and development of a Star race of Drosophila melanogaster*.—A race of Star has been crossed for several generations to a highly inbred, vigorous wild strain of *D. melanogaster*. Preparations have been made of eggs allowed to develop at  $25 \pm 0.1^\circ\text{C}$  for various definite periods up to and including 48 hours. The arrest in development of homozygous Star eggs occurs at a relatively late stage with larval jaws, suprapharyngeal musculature and other larval organs formed. It is planned to demonstrate preparations of normal and homozygous Star eggs.

SPENCER, WARREN P., College of Wooster, Wooster, O.: *Drosophila virilis americana, a new sub-species*.—A line of flies descended from an impregnated wild female taken in a trap in open woods in Wayne County, Ohio, proves to be a new sub-species of *Drosophila virilis*. *Drosophila virilis americana* differs from *Drosophila virilis virilis* in the following characters: more fusiform body, darker body color, larger eye, finer eye pile, broader carina, heavier cloud on posterior cross-vein, and reddish rather than gray pupa case. In the former pupation occurs in the culture medium rather than on the sides of the culture vial. *Americana* etherizes very rapidly in contrast to the other sub-species.—The cross-matings give a few hybrid offspring. The mating, *virilis* female



by *americana* male, is more often successful than the reciprocal. When *virilis* females are impregnated by *americana* males about 2 percent of the eggs develop. The hybrids, both males and females, are partially fertile when crossed inter se or back to either parent sub-species. The  $F_1$  hybrid resembles *americana* more closely than *virilis*.  $F_2$  and back-cross experiments indicate that all of the sub-specific characters observed depend upon multiple factors. —Salivary chromosomes of the hybrid larva show loose pairing in certain regions and three major inversions, each in a different chromosome pair. In morphological and physiological characters the two sub-species differ more than do *D. pseudoobscura* and *D. miranda*, but in terms of salivary chromosome structure and fertility of the hybrids they appear to be more closely related.

SPENCER, WARREN P., College of Wooster, Wooster, O.: *Multiple alleles at the bobbed locus in populations of Drosophila hydei*. —Of 27,805 flies from a population of *Drosophila* breeding on a citrus dump near Azusa, Southern California, 99.3 percent were *D. hydei*. 3.1 percent of the females were classified as distinctly bobbed. Further analysis included single matings of 118 wild males to females from standard bobbed stocks, 54 virgin bobbed females reared from collected pupae to standard bobbed males, and subsequent backcross and  $F_2$  matings. —It was shown that: (a) the population contained an extremely complex series of multiple alleles ranging from homozygous lethal types at one end to a group which in homozygous condition gave no visible bobbed characters but which could be seriated in their reactions with strong bobbed alleles; (b) the phenotypic complex included bristle length, abnormal abdominal sclerites, length of larval-pupal life, time of maturing after emergence, sterility and lethal effects; (c) some alleles acted more strongly on one phenotypic character, others on another, and their heterozygous combinations varied toward wild type; (d) most bobbed females tested carried two different and easily separable bobbed alleles; (e) the series showed no indication of complete dominance. —There is considerable evidence that *D. hydei* populations generally carry bobbed alleles. The author has found bobbed in wild stocks collected from San Gabriel Canyon and Providence Mountains, California, Wooster, Ohio (1925 collection), Huntington, New York, and Coffeyville, Kansas. In November, 1937, from a large population of *D. hydei* near Wooster, Ohio approximately 10,000 flies have been collected. A sample of 1575 females examined showed 3.8 percent easily classified as bobbed.

STADLER, L. J., U. S. Department of Agriculture and University of Missouri, Columbia, Mo.: *On the possibility of disentangling by spectrological means the complex of genetic effects induced by radiations*. —Genetic alterations induced by penetrating radiations include translocations, deficiencies, and point mutations. In addition, in maize, "germless seeds" are produced, resulting from alterations (induced by pollen treatment) which prevent the

normal development of the embryo. These may be designated "dominant lethals," though as in other postulated dominant lethals there is no assurance that the underlying alteration is genic or even chromosomal.—Many attempts have been made to interrelate some or all of these effects, so as to derive them from some single basic change produced by the radiation. On the other hand it is often argued that some of the effects (that is, deficiencies and mutations) must be distinct in origin and nature. All of these effects, however, are found in all experiments suitable for their demonstration within the X-ray to gamma-ray range.—Comparison of X-ray and ultra-violet effects in maize indicates that at least three of the four are spectrologically separable. Ultra-violet rays failed to affect the translocation rate, while it greatly increased the frequency of germless seeds, deficiencies, and mutations. As previously shown (STADLER and SPRAGUE 1936) this contrast might be incidental to dosage, but later studies (with J. W. CAMERON) of the X-ray dosage relation show that this is not the case. The ultra-violet ray effects on germless seeds and deficiencies have distinctive spectral characteristics (see STADLER and UBER, these abstracts). Thus the alterations resulting in translocation are separable from those resulting in the other three effects, and those producing germless seeds are separable from those producing deficiencies. The spectral correspondence of point mutation with deficiency and the germless seed effect remains to be determined.

STADLER, L. J. and UBER, FRED M., University of Missouri and U. S. Department of Agriculture, Columbia, Mo.: *Preliminary data on genetic effects of monochromatic ultra-violet radiation in maize.*—Pollen was treated with monochromatic radiations of wave-lengths 235, 238, 240, 248, 254, 265, 280, 297, 302, 313 and 365 $\mu$ . Absorption determinations on pollen grain contents showed that penetration does not vary widely with wave-length in this range. Tolerance (maximum dose permitting seed production) varied from  $1.2 \times 10^3$  ergs/mm<sup>2</sup> at 235 $\mu$  to more than  $2.5 \times 10^6$  ergs/mm<sup>2</sup> at 365 $\mu$ . There were four fairly distinct tolerance levels: (1)  $1.2 \times 10^3$  ergs/mm<sup>2</sup> for  $\lambda$ 235–240, the limiting factor in this range being clumping of pollen; (2)  $1.2 \times 10^4$  ergs/mm<sup>2</sup> for  $\lambda$ 248–280; (3)  $1.3 \times 10^5$  ergs/mm<sup>2</sup> for  $\lambda$ 297 and 302; and (4) more than  $10^6$  ergs/mm<sup>2</sup> for  $\lambda$ 313 and 365, at which the highest doses applied had no effect on seed production. Deficiencies (marked by loss of *A*, *Pr*, *Su Wx*, and *Y* in endosperm) were induced in large numbers by all wave-lengths of the range 235–302 $\mu$ . Throughout this range fractionals as well as entire endosperm deficiencies were numerous. Longer wave-lengths had no appreciable effect on the frequency of deficiency. The frequency of "germless" seeds was increased markedly by radiation of wave-lengths 280 $\mu$  and shorter but not materially affected by longer wave-lengths, even at much higher dosages. Thus,  $\lambda$ 297 and 302, which are tolerated in heavy doses, are highly effective in inducing deficiencies and have little or no effect in producing germless seeds. The results suggest that the three phenomena are the result of induced alterations which have distinctive spectral characteristics.

STEELE, DEWEY G., Connecticut State College Storrs, Conn.: *A colorimetric method for the quantitative study of melanin in feathers*.—Colorimetric methods have been applied to the quantitative study of melanin in the feathers of several color types of the chicken. Consistent and reproducible results have been obtained by this procedure and they will be shown in tabular form. They will demonstrate the quantitative effect of several so-called qualitative color factors. The steps in the procedure are as follows: (1) Obtaining the sample.—All feathers should be obtained from the same region of the bird. In this study only dorsal wing coverts from the region of the wing bow have been used.—(2) Washing the feather sample, followed by drying for 24 hours in an oven at 103°C, then weighing.—(3) Digesting the feathers by boiling the sample for 15 minutes in 100 cc of 2.5 percent NaOH. Boiling is done in a flask connected with a reflex condenser. Immediately following the digestion process the contents of the flask are cooled by immersing the flask in cold water.—(4) A small portion, such as 1 cc or 5 cc of the digested sample is diluted with sufficient 2.5 percent NaOH to give the desired concentration for the test solution. Concentrations ranging from 1 gram of feathers in 1000 cc of the caustic solution to 1 gram in 8000 cc have been studied, and the results show that the less dilute test samples may be more favorable for detecting small differences than those at the other extreme.—(5) The light blocking power of the test sample is measured in an electrometric colorimeter and the readings are expressed in terms of microamperes. The instrument should be standardized before each reading, and if this is properly done duplicate readings may be obtained by different operators.—The method does not yet offer the means for determining the absolute quantities of melanin in feathers but it does provide a means for determining the relative amounts of pigment in comparable color types.—The general procedure should be applicable to other animals where the same problems are being studied.

STERN, CURT, University of Rochester, Rochester, N. Y.: *The innervation of setae in Drosophila*.—By means of the methylene blue technique of intravital staining it can be demonstrated that on the thorax of *Drosophila* underlying the pattern of micro- and macrochaetae (hairs and bristles) there exists a pattern of peripheral nerve cells. They are bipolar cells situated below the hypodermis. From each cell one short fiber is sent to the base of a seta while the second, long fiber, joins up with similar long fibers from other cells into nerves which lead to the central nervous system. Bristles, in contrast to hairs, are innervated by a large, instead of a small, cell. An interesting detail of the nerve pattern is the fact that the nerve cell belonging to the anterior dorso-central bristle combines with a different nerve-trunk than the nerve cell of the posterior dorsocentral. The former sends its axon toward an anterior nerve, the latter toward a posterior. A complete association seems to exist between the typical nerve cells and the setae so that each seta is innervated, and each nerve cell is connected with a seta. This is true not only for normal flies but

also for mutant races with increased (Hairywing, hairy) or decreased number of setae (achaete, scute, Dichaete). In mosaic spots of hairy, on flies heterozygous for this gene, every seta again is innervated. These studies are undertaken as a step in an analysis of bristle pattern.

STONE, W. S., University of Texas, Austin, Tex.: *The Y chromosome and pigment formation in Drosophila melanogaster*.—The Y chromosome has been shown to have a positive stabilizing effect on the amount of eye pigment in the case of white and Plum mottles (GOWEN and GAY, SCHULTZ, DUBININ, and others). This influence on pigmentation is a more general one and extends to body color as well. An "unstable gene" for gray in scute<sup>s</sup> stock 635q produces flies mosaic for gray-yellow, XY males are predominantly gray with a few yellow splotches, and the XO or X-XR (hyperploid for *B-bb* region) practically yellow with a few gray bristles and hairs; the right end of the X lacks this stabilizing effect. The Y chromosome has a non-specific factor which stabilizes and normalizes pigment formation.

STONE, W. S., and GRIFFIN, A. B., University of Texas, Austin, Tex.: *"Free" centromeres in Drosophila melanogaster*.—In *D. melanogaster* two stocks have been obtained in each of which the centromere of one chromosome has associated with it a few genes only. These stocks are translocation X-IV 10, with region 1A (broken to the left of yellow) attached to the IV at 102A1, and a deletion in IV with the region between 101F1 and 102F3 missing. Hyperploids for each of these small chromosomes are normal. Somatic tissues of females homozygous for the translocation appear to have only three pairs of chromosomes. However, the small chromosomes can be seen occasionally in salivary gland nuclei, if a careful search is made. When present as hyperploids, these small chromosomes constitute "free" centromeres, which would afford an opportunity of increasing the chromosome number through translocation, just as a free Y chromosome could do (STURTEVANT and TAN and others).

STURTEVANT, A. H., California Institute of Technology, Pasadena, Cal.: *The homologies of the chromosome arms of different species of Drosophila*.—There are three available methods of studying the homologies of the chromosomes of species of *Drosophila*. In the case of species that can be hybridized it is possible to study the allelomorphism of mutant genes (as in *D. melanogaster* and *D. simulans*), or to study the conjugation of the salivary chromosomes of the hybrids (as in the above case and in that of *pseudoobscura* and *miranda*). But most species cannot be hybridized, and only the third method is available, namely, the identification of mutant genes by their phenotypic effects. Charts will be presented showing the results of such a comparison. The table on the following page is a condensed summary:

| Species              | Equivalent Chromosome Arms |      |      |       |       |    |
|----------------------|----------------------------|------|------|-------|-------|----|
| <i>melanogaster</i>  | X                          | II L | II R | III L | III R | IV |
| <i>simulans</i>      | X                          | II L | II R | III L | III R | IV |
| <i>pseudoobscura</i> | X L                        | IV   | III  | X R   | II    | V  |
| <i>miranda</i>       | X L                        | IV   | X 2  | X R   | II    | V  |
| <i>virilis</i>       | X                          | IV   | V    | III   | II    | VI |

The success of the method indicates that in no case have large sections been transferred from one arm to another. X-ray studies show that such transfers occur frequently. It may be concluded that the products practically never survive under natural conditions, even in the time periods concerned in very extensive species differentiation.

WARREN, D. C., Kansas State College, Manhattan, Kan.: *Mapping the genes of the fowl*.—Four linkage groups are now known in the fowl. The sex-linked group has 6 factors while the 3 autosomal groups are comprised of 4, 3, and 2 factors respectively.—Six other factors are now fairly well established as markers of additional chromosomes, all having been shown to segregate independently of each other as well as of the 4 established linkage groups. In the case of the 6 groups with a single factor, the numbers involved are large enough to make it improbable that any of them will be found to belong to the same group. The available data now provide markers for 10 chromosomes in the fowl.—The known genes in the sex chromosome are barring, slaty shank, head streak, silver, late feathering and light down. The first described autosomal linkage group carries the factors, rose comb and creeper. The next group consists of crest, dominant white, frizzling, and fray. The fourth group has the factors silkie, flightless, and naked neck. The 6 independently segregating factors are rumplessness, polydactyly, pea comb, white skin, leg feathering, and blue plumage.

WETTSTEIN, FR., Kaiser Wilhelm Institute für Biologie, Berlin Dahlem, Germany: *Hemiploid forms and some problems of polyploidy*.—By experimental investigations within the last decades we are informed about origin and characters of a number of experimentally produced polyploid forms among plants. Cytological investigations show that within many genera of flowering plants the chromosome numbers of species form arithmetical series. They are designated as cytological polyploid series. This implies the assumption that these species have arisen by polyploidy in one of the ways known from experimental polyploid forms.—To support this conception two proofs are still necessary. Firstly there has to be demonstrated that a natural species with high chromosome number is actually a polyploid form. Secondly it has to be proved that experimental polyploid forms also may show the same characters observed in natural polyploid ones. In both cases we supply examples out of

our research work with mosses.—MUNTZING has already derived from the cross *Galeopsis pubescens* by *G. speciosa* a tetraploid form equivalent to the tetraploid wild form of *G. Tetrahit*. From the wild form of a moss, *Physcomitrium piriforme* ( $n=36$  and  $2n=72$ ) we obtained two forms with half the haploid number  $n/2=18$ , the so-called hemiploids. Recombining both, we again get the normal haploid wild form. This proves that the wild form of *P. piriforme* should be regarded as a polyploid.—By regeneration of the diploid sporophytes of *Bryum caespitium* we obtain a dioecious bivalent race with large cell volume, gigas characters and low fertility. Due to a strange regulation process in the course of eleven years the large cell volume has changed to a low one, the gigas characters have disappeared, the fertility has become quite normal. In spite of this the number of the chromosomes remains unchanged. In this way a polyploid race with the normal characters of a univalent race has resulted.

WHITING, ANNA R., University of Pennsylvania, Philadelphia, Pa.: *Mutant body colors in Habrobracon and their mosaics*.—In *Habrobracon* wild type have honey-yellow body color with a pattern of black varying in extent and intensity inversely as the temperature. Extremities (antennae, feet, ovipositor sheath, and wings) have black pigment which remains constant at differing temperatures. Three linked mutant genes, all recessive, affect body color. Honey (*ho*) eliminates black in all parts at any temperature, leaving almost uniform honey color. Lemon (*le*) reduces yellow to pale lemon and limits extent of black pattern but leaves extremities dark except that basal antennal segments are bright yellow (a dominant trait). Black (*bl*) intensifies and extends the black pattern and reduces the restricted light areas to pale yellow. In double recessives black fails to produce or extend a black pattern but causes a suffused sootiness with reduction of yellow. In lemon-honey the general tone is pale cream while in black-lemon-honey it is sooty cream. It is possible that these facts are analogous to those concerned with coat colors in Mammals as analyzed by WRIGHT; that the formation of black pigment is dependent on two substances (1 and 2), only one of which (1) is necessary for yellow. Relative excess of substance 2 results in more black and exhausts substance 1, tending to make "yellow" regions sooty cream. The mutant body colors are relatively little temperature affected. In mosaics each genetically different region is autonomous for body color. Colored paintings have been made with aid of a grant from the Penrose Fund of the American Philosophical Society.

WHITTINGHILL, M., California Institute of Technology, Pasadena, Cal.: *Oögonial crossing over in Drosophila melanogaster*.—The occurrence of oögonial crossing over may be demonstrated by X-raying marked females homozygous for the asynaptic factor, C3G, which normally prevents almost all crossing over. About 200 such females heterozygous for the mutants *sc ec cv ct v s f car* were X-rayed with 2500 r or 3500 r and then mated individually to

"x<sup>9</sup>" multiple recessive males. Each mating was transferred through a series of five cultures until 23 days after raying. Sixteen crossover offspring were found, nine of which could have been due only to the occurrence of crossing over in oögonial cells followed by the multiplication of the crossover chromosomes. These nine crossovers came from eggs laid by four females during the 10th to 19th days after X-irradiation. No female produced more than one class or two complementary classes of crossovers. In each of the four regions for which crossovers were found they occurred in groups almost as often as singly per family, whereas in untreated material GOWEN (1929) found crossovers singly only. It is obvious that in females lacking the asynaptic factor oögonial crossing over, if present naturally or by induction, is obscured as such by the usual meiotic crossing over. Hence an increase in the number of crossovers recovered may be due to *either* of two causes: an actual, pronounced increase in the occurrence of meiotic crossing over; or the earlier occurrence of an occasional mitotic crossing over in gonial cells.

WILLIAMS, L. F., and WILLIAMS, G. S., U. S. Department of Agriculture, Urbana, Ill.: *The use of prime types in linkage studies in the soybean*.—A cross between Hahto and Type 116, a strain descended from a seed irradiated by Dr. J. T. BUCHHOLZ, produced plants having 44 percent of the ovules undeveloped and approximately 50 percent shrivelled pollen. In the F<sub>2</sub> generation about half of the plants had good pollen and normal seed development and half showed  $\pm 50$  percent bad pollen and  $\pm 50$  percent undeveloped ovules. This indicated the existence of reciprocal translocations or prime types in the parent strains. F<sub>3</sub> pedigrees from both types of F<sub>2</sub> plants support this.—The F<sub>1</sub> has an average of 44 percent undeveloped ovules but a necessary correction, due to the fact that pods in which all ovules fail to develop are dropped raises the percentage of undeveloped ovules to 50.8 percent. This type of ovule failure differs, in kind and in distribution in the pod, from the environmental type of seed abortion studied by WOODWORTH and others.—Since the failure of half of the ovules produces a characteristic appearance of many one-seeded pods, pedigrees can be classified for plants homozygous and heterozygous for the prime types without pollen or chromosome examination. This should be very useful in future linkage studies in the soybean. Plants heterozygous for prime types can thus be identified by examination of chromosomes, pollen or pods.—The parents differed by eleven known genes but due to epistasis and gene interaction, data for only eight are available. Of these *L*, *G*, *V*, *R* and *Fl*, clearly are neither linked among themselves nor with these prime types. One gene, *I*, is apparently linked with these prime types, giving  $\pm 14$  percent crossing over in a count of 284 individuals.

WOOLLEY, GEORGE W., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.: *A mutation causing the loss of one pair of palatal ridges in the house mouse, Mus musculus*.—An examination of a number of strains of mice indicates that the common number of palatal ridges is eight. 1-3 lie be-

tween the incisor and first molar teeth and are unpaired. 4-8 are inter-molar and paired. Families in which the individuals have only four paired palatal ridges have been found in the Jackson Laboratory C57 black strain of mice. The results of crosses between mutant and normal individuals and their offspring demonstrate that the ridge loss is due to a recessive mutation. Evidence has been secured showing the fifth, or second paired, ridge to be absent in the mutant form. It is possible to determine the ridge number any time after birth, and in live mice.





# THE PRODUCTION OF MUTATIONS IN DROSOPHILA WITH NEUTRON RADIATION

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Received September 30, 1937

EVER since the discovery of neutrons in 1932 by Dr. J. CHADWICK of the Cavendish Laboratory, Cambridge University, difficulties have stood in the way of the use of neutrons in the biological field. In the first place, it has been difficult to produce them in sufficient intensities. Secondly, nearly all of the neutron sources that might have been used not only emit neutrons of very heterogeneous energies, but also emit intense gamma radiation whose wave lengths vary through wide ranges. Moreover, the gamma radiation from most neutron sources is completely inseparable from the neutron radiation, so that the biological effects due to the neutrons and to the gamma rays cannot usually be separated by the use of gamma-ray control experiments. Finally, measurements of the intensities of neutron beams, or even determinations of their orders of magnitude, are attended by serious experimental difficulties. Through physical research, these drawbacks have been partly overcome, although there still remains much to be accomplished. Meanwhile, great care must be taken both in the performance and in the interpretation of biological experiments with neutrons.

Very few experiments with neutrons in the field of biology have been reported. LAWRENCE and LAWRENCE (1936) have found that fast neutrons are approximately ten times as biologically effective as X-rays in altering the blood picture of rats. ZIRKLE and AEBERSOLD (1936) performed experiments to determine the comparative effects of X-rays and fast neutrons on wheat seedlings. Fast neutrons were found to be about twenty times more effective than the equivalent dosage of hard X-rays in retarding growth in wheat seedlings.

The only genetic experiment with neutrons that has come to the attention of the writers is that of WHITING (1936) on the production of dominant lethals in the parasitic wasp, *Habrobracon*. The data presented however, as the author states, are meager.

The possible efficacy of neutrons in causing mutations was suggested by one of us in a recent paper (LOCHER 1936). The genetical aspect of the present problem was carried out under the guidance and suggestions of Dr. EDGAR ALTENBURG to whom we wish to express our sincere thanks and appreciation. We wish to thank the AMERICAN ONCOLOGIC HOSPITAL at Philadelphia for the use of the 4-gram radium bomb, through the courtesy

of Dr. G. M. DORRANCE, the director, and Dr. F. E. ULRICH, of the RICE INSTITUTE, for working out the probable errors in the results.

A preliminary account of this experiment has been reported in *Nature* (NAGAI and LOCHER 1937).

#### PRODUCTION OF NEUTRONS

Neutrons, like alpha particles, exist only in atomic nuclei and must be ejected from them if they are to be used. Neutrons for this experiment were obtained with the "radium bomb" of the AMERICAN ONCOLOGIC HOSPITAL, (NEWCOMET and HUGHES 1935), by allowing part of the gamma radiation from 4 grams of radium to pass through 485 grams of beryllium metal in a cylinder 8.0 cm in diameter and 9.0 cm in height. The radium holder, containing eighty 50-mg needles, was put in contact with one end of the beryllium cylinder, inside the massive lead bomb.

Gamma-rays whose energies exceed 1.6 MEV (millions of electron volts) can disintegrate beryllium nuclei, with the emission of neutrons, by the "photo-nuclear effect"; the neutrons thus obtained are sometimes called "photo-neutrons." In the present case, two gamma-ray lines of the RaC—RaC' spectrum (1.76 MEV and 2.20 MEV, respectively) are capable of effecting this disintegration. The former line predominates in intensity. The kinetic energies of the neutrons are, therefore,  $1.76 - 1.6 = 0.16$  MEV (or 160 kilovolts), and  $2.20 - 1.6 = 0.60$  MEV (or 600 kilovolts), respectively. The total emission (in all directions) from the present source is calculated to be, roughly, 200,000 neutrons per second.

It is to be expected that the chief biological effects of fast neutrons will arise from the ionization along paths of recoil protons (hydrogen nuclei) that they eject by elastic collisions. The maximum ranges, in air, of protons ejected by unscattered neutrons from the present source are, according to the Cornell University energy-range curves of 1937, 0.22 cm and 1.1 cm, respectively. In water, the corresponding ranges are, approximately, 2.2 microns and 11 microns, respectively. Most of the recoil protons will, of course, have much smaller ranges, since their paths are oblique to the directions of incidence of the impinging neutrons.

Lead is rather transparent to fast neutrons, since the fraction of the kinetic energy lost by a neutron at each scattering decreases with the atomic number of the scattering element (1.9 percent, maximum, for lead, as compared with 100 percent maximum, for hydrogen). On the other hand, the scattering cross-section (or the probability of scattering) of the fast neutrons increases slowly with atomic number. The action of the thick lead shield provided by the wall of the radium bomb is, then, to introduce considerable diffusion of the (fast) neutron beam, without great absorption of energy from it. The lead intervening between the source and the point

of observation produces scattering-out of the neutrons, but all other lead in the vicinity produces scattering-in.

Figure 1 shows the arrangement of the neutron source and flies during irradiation. Two groups of flies, (F) and (S), were simultaneously irradiated with fast and with slow neutrons, respectively. The flies were placed as close to the beryllium block as was compatible with the retention of an adequate thickness of lead for the absorption of the gamma radiation.

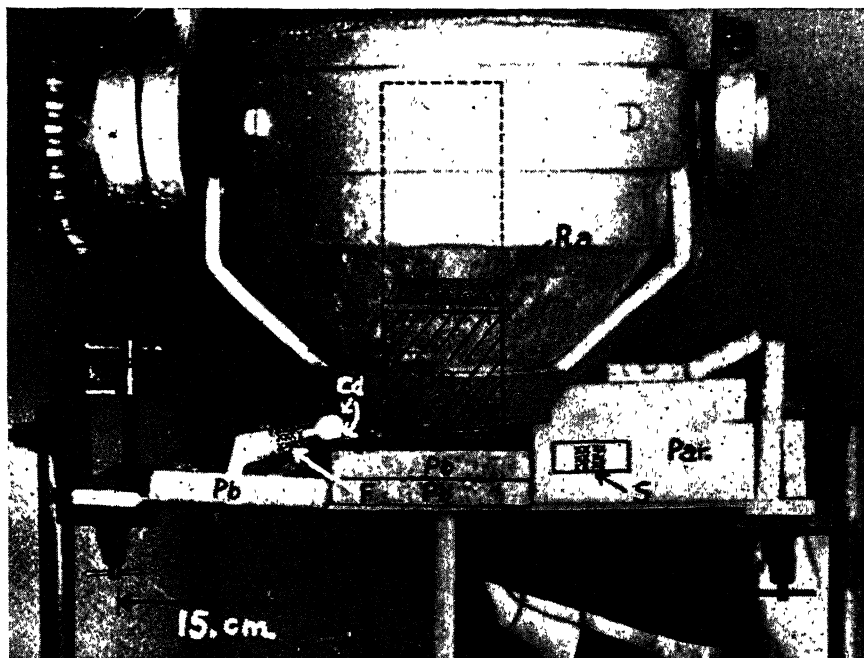


FIGURE 1.—Arrangement of apparatus during irradiation of flies with photo-neutrons. The radium (Ra) and beryllium (Be) are in a central hole of the lead bomb. The flies (F), irradiated with fast neutrons, are protected from very slow neutrons by a cadmium screen (Cd). A paraffin box (Par.) slows down the neutrons, by scattering, for irradiation of another group of flies (S) with slow neutrons. Lead blocks (Pb) and the lead and steel of the radium bomb absorb most of the  $\gamma$ -rays from the radium.

The distance of the perpendicular from the "line of centers" of the radium bomb to the mid-position of the flies in the vials was 12.6 cm. The distance from the center of the beryllium source to the mid-position of the flies was 11 cm. A cadmium filter 0.023 cm in thickness was used to filter out the very slow neutrons that may reach the flies as a result of scattering in beryllium and paraffin. The scattering in the beryllium, however, has been shown to be negligible. The flies thus protected by the cadmium filter received the "fast" neutron treatment.

The "slow" neutron flies were placed at the same distance from the

beryllium source in a paraffin box which weighed 4.5 pounds. The paraffin serves to slow down the neutrons by multiple scattering with hydrogen; its use for this purpose is general practice in neutron experiments. The paraffin facing the beryllium source was 1.2 cm in thickness, while that on all other sides of the box presented a thickness of at least 2.4 cm to neutrons incident from the beryllium source or scattered into the box by the lead of the bomb. The side of the box farthest from the bomb was about 5.0 cm thick and served as a backward-scatterer for the neutrons. This arrangement was chosen for its relatively high yield of slow neutrons, on the basis of a previous series of experiments with the production of artificial radioactivity in a special Geiger-Müller counter with a silver cathode. The distance of the "slow" neutron-treated flies from the beryllium source was approximately the same as that given for the "fast" neutron flies.

The terms "fast neutrons" and "slow neutrons" are, of course, only relative, in the same sense that "hard X-rays" and "soft X-rays" are relative terms. The fast neutrons used here are liberated with two distinct kinetic energies which are probably not greatly altered before the particles reach the flies. The slow neutrons, however, undoubtedly have a continuous range of energies that extend from those of the unscattered neutrons to zero, mainly as the result of scattering in the paraffin. The total number of fast neutrons in the "slow" group might be roughly 25 percent as great as in the fast neutron group.

Since some gamma radiation from the radium accompanies the neutrons, and since exact physical measurements of its biological effectiveness cannot be made, gamma-ray controls are necessary, especially in view of the fact that gamma-rays cause mutations (HANSON and HAYS 1928; HANSON and WINKLEMAN 1929). A paraffin "dummy" of the same dimensions as those of the beryllium source was used in place of the latter. It should give about the same amount of scattering of the gamma radiation as does the beryllium, without emission of an appreciable number of neutrons. Two sets of gamma-ray controls were run; one set with the flies in paraffin and the other in air. The latter should have had a little more gamma radiation than the one kept in paraffin; however, the difference in effect between the two should be almost negligible.

Ionization measurements of gamma-ray intensity at the position of the flies, both in neutron-irradiated and gamma-ray control groups, were made. A small ionization chamber (12 cc volume, with argon at 10 atmospheres) was connected through a semi-flexible cable to a vacuum-tube electrometer; the latter was used as a null-method instrument. The ionization current measured at the position of the flies was 50 micro-micro-amperes as compared with a current of 1.91 micro-micro-amperes due to ionization

by 1.00 mg of radium in a single needle 10 cm from the center of the chamber. The radiation on flies would therefore, be  $50/1.91$  or 26.2 mg Ra equivalent at 10 cm, or at 1 cm, 0.262 mg Ra equivalent. Taking TAYLOR'S (1936) value of 8.6 roentgens per mg Ra at 1 cm from a point source of Ra, the radiation on the flies per hour would be  $8.6 \times 0.262 = 2.25$  roentgens. Thus, the flies exposed to 36 hours of irradiation (the usual treatment in these experiments) received  $2.25 \times 36$  or 81 roentgens.

Certain classes of slow neutrons may be measured, but it is not as yet possible to measure the intensity of either the slow or the fast neutrons falling on the flies in these experiments. We hope to measure the neutron flux from the source we used by a new method now under development.

#### PRODUCTION OF MUTATIONS

To determine the effect of neutrons on the mutation rate, adult eosin males of *Drosophila melanogaster* were treated and lethals in the sex chromosome looked for. The lethals were detected by MULLER'S CIB method. One treated male was used per bottle in the  $P_1$ , and the  $F_1$  Bar females were reared in vials. The lethals were located with regard to  $w^e v f$ . For convenience a lethal in the region between  $w^e$  and  $v$  is indicated as I, and between  $v$  and  $f$  as II in the tables. The symbols  $I^s$  and  $II^s$  indicate semi-lethals in these regions. Inv. indicates an inversion.

The eosin stock flies used in all of the experiments in any particular series were of the same kind. They were taken from several different bottles, mixed well, and then divided into three separate vials for each of the four experiments in each series. The three groups of flies were, namely, for (1) the neutron-treated, (2) the corresponding gamma-ray controls, and (3) the non-irradiated controls which did not receive any kind of radiation. They all received identical handling except during treatment period. The neutron-treated flies and the corresponding gamma-ray control flies were not exposed to their respective radiations at the same time. They were usually exposed alternately, for equal intervals of 3 to 8 hours.

Three series of experiments were carried out with the Ra-Be source of neutrons previously described. The first series, or the preliminary series, consisted of experiments A, B, C, D; the second, experiments E, F, G, H; and the third series, experiments I, J, K, L. The data for each series will be considered separately, then the summarized data will be given.

The results of the first series of experiments are shown in table 1. Experiments A and C met with accident and no data were obtained from them. The gamma-ray controls of experiment D died (in transport from Swarthmore to Houston). The dosage of neutrons in these experiments was low (3 to 9 hours), since it was not yet known what the maximum dosage was that the flies could tolerate (or that could be administered under the

TABLE 1

| EXP. NO. | TREATMENT     | NO. HRS. | NO. MALES MATED | NO. MALES STERILE | NO. MALES TESTED | NO. F <sub>1</sub> | STERILITY NO. % | NO. F <sub>1</sub> TESTED | LETHALS NO. % | CLASSIFICATION AND LOCATION FROM DIFF. MALE | MUTATION RATE |
|----------|---------------|----------|-----------------|-------------------|------------------|--------------------|-----------------|---------------------------|---------------|---|---------------|
| B        | Fast Neutrons | 6        | 23              | 2                 | 21               | 993                | 42 4.22         | 951                       | 6 .63         | 4(II, I, II, I) 2(II <sup>a</sup> , II)     | 1 in 158.5    |
| XB       | Gamma rays    | 6        | 17              | —                 | 17               | 741                | 39 5.26         | 702                       | 4 .56         | 2(II <sup>a</sup> , I)                      | 1 in 175.5    |
| CB       | No Radiation  | —        | 18              | 1                 | 17               | 729                | 48 6.58         | 681                       | 1 .15         | 1(II)                                       | 1 in 681.0    |
| D        | Fast Neutrons | 9        | 5               | 1                 | 4                | 226                | 10 4.42         | 216                       | 1 .46         | 1(II <sup>a</sup> )                         | 1 in 216.0    |
| XD       | Gamma rays    | 9        | —               | —                 | —                | —                  | —               | —                         | —             | —   | —             |
| CD       | No Radiation  | —        | 12              | —                 | 12               | 593                | 34 5.73         | 559                       | 3 .53         | 3(II <sup>a</sup> , I, II)                  | 1 in 186.3    |

TABLE 2

| EXP. NO. | TREATMENT     | NO. HRS. | NO. MALES MATED | NO. MALES DEAD | NO. MALES STERILE | NO. MALES TESTED | NO. F <sub>1</sub> | STERILITY NO. % | NO. F <sub>1</sub> TESTED | LETHALS NO. % | CLASSIFICATION AND LOCATION FROM DIFF. MALE   | MUTATION RATE |
|----------|---------------|----------|-----------------|----------------|-------------------|------------------|--------------------|-----------------|---------------------------|---------------|---|---------------|
| E        | Slow Neutrons | 16       | 12              | —              | 1                 | 11               | 601                | 68 9.84         | 623                       | 1 .16         | 1(I)  | 1 in 623.0    |
| XE       | Gamma rays    | 16       | 18              | —              | 1                 | 17               | 863                | 115 13.32       | 748                       | 3 .40         | 3(II, I, I)   | 1 in 249.3    |
| CE       | No Radiation  | —        | 19              | 1              | 2                 | 16               | 729                | 49 6.72         | 680                       | 3 .44         | 3(II, I, I)   | 1 in 256.0    |
| G        | Fast Neutrons | 16       | 21              | —              | —                 | 21               | 1969               | 87 4.42         | 1882                      | 18 .96        | 3(II <sup>a</sup> , II, II)<br>2(II <sup>a</sup> , II)<br>2(I, II)<br>2(II, I)<br>2(I, I) | 1 in 104.5    |
| XG       | Gamma rays    | 16       | 22              | —              | 1                 | 21               | 1658               | 104 6.27        | 1594                      | 6 .39         | 5(II <sup>a</sup> , II, II, I, I)<br>6(I, II, II <sup>a</sup> , II <sup>a</sup> , II, I)  | 1 in 259.0    |
| CG       | No Radiation  | —        | 9               | —              | —                 | 9                | 485                | 37 7.63         | 448                       | — .00         | —   | 0 in 448.0    |
| H        | Fast Neutrons | 40       | 11              | —              | 1                 | 10               | 794                | 69 8.69         | 725                       | 6 .83         | 4(II, II, ? I) 2(I, II)   | 1 in 120.8    |
| XH       | Gamma rays    | 40       | —               | —              | —                 | —                | —                  | —               | —                         | —             | —   | —             |
| CH       | No Radiation  | —        | 8               | —              | 1                 | 7                | 264                | 15 5.68         | 249                       | —             | —   | 0 in 249.0    |
| CF       | No Radiation  | —        | 6               | —              | —                 | 6                | 349                | 32 9.17         | 317                       | —             | —   | 0 in 317.0    |

TABLE 3

| EXP. NO. | TREATMENT     | NO. HRS. | NO. MALES MATED | NO. MALES DEAD | NO. MALES STERILE | NO. MALES LOST | NO. MALES TESTED | NO. F <sub>1</sub> | STERILITY % | NO. TESTED | LETHALS NO. | LETHALS % | CLASSIFICATION AND LOCATION |   |                          | MUTATION RATE |
|----------|---------------|----------|-----------------|----------------|-------------------|----------------|------------------|--------------------|-------------|------------|-------------|-----------|-----------------------------|---|--------------------------|---------------|
|          |               |          |                 |                |                   |                |                  |                    |             |            |             |           | FROM DIFF. MALES            | FROM SAME MALES   | FROM SAME MALES          |               |
| I        | Slow Neutrons | 36       | 18              | —              | 1                 | —              | 17               | 632                | 64          | 10.1       | 568         | 4         | .70                         | 4(I <sup>a</sup> , II <sup>a</sup> , I <sup>a</sup> , II) | —                        | 1 in 142.0    |
| XI       | Gamma rays    | 36       | 15              | —              | 5                 | 5              | 5                | 98                 | 21          | 21.4       | 77          | —         | —                           | —   | —                        | 0 in 77.0     |
| CI       | No Radiation  | —        | 13              | —              | 1                 | 2              | 10               | 573                | 40          | 6.98       | 533         | 4         | .75                         | 4(I <sup>a</sup> , II, I, I <sup>a</sup> )                | —                        | 1 in 133.2    |
| J        | Slow Neutrons | 36       | 22              | 2              | 6                 | —              | 14               | 368                | 55          | 14.9       | 313         | —         | —                           | —   | —                        | 0 in 313.0    |
| XJ       | Gamma rays    | 36       | 19              | 1              | 6                 | 1              | 11               | 406                | 107         | 26.3       | 299         | —         | —                           | —   | —                        | 0 in 299.0    |
| CJ       | No Radiation  | —        | 22              | 2              | 3                 | 1              | 16               | 1155               | 135         | 11.6       | 1020        | —         | —                           | —   | —                        | 0 in 1020.0   |
| K        | Fast Neutrons | 36       | 22              | —              | 4                 | —              | 18               | 856                | 122         | 14.2       | 734         | 8         | 1.09                        | 3(II, I, II)  | 3(I, II, II)<br>2(II, I) | 1 in 91.7     |
| XK       | Gamma rays    | 36       | 21              | —              | 4                 | 1              | 16               | 1093               | 135         | 12.2       | 958         | 3         | .31                         | 3(II, I, I)   | —                        | 1 in 319.3    |
| CK       | No Radiation  | —        | 13              | —              | 1                 | —              | 12               | 839                | 129         | 15.3       | 710         | 1         | .14                         | 1(II <sup>a</sup> )                                       | —                        | 1 in 710.0    |
| L        | Fast Neutrons | 36       | 22              | —              | 2                 | —              | 20               | 1126               | 155         | 13.7       | 971         | 12        | 1.23                        | 6(I, II, II, I, I, I)                                     | 2(II, II)<br>2(Inv, I)   | 1 in 80.9     |
| XL       | Gamma rays    | 36       | 24              | 1              | 4                 | —              | 19               | 1311               | 183         | 13.9       | 1128        | 7         | .62                         | 7(I, II <sup>a</sup> , II, I, I, I, I)                    | 2(II, II)                | 1 in 161.1    |
| CL       | No Radiation  | —        | 26              | 1              | 4                 | —              | 21               | 930                | 115         | 12.3       | 815         | 4         | .49                         | 4(I, v, II <sup>a</sup> , II)                             | —                        | 1 in 203.7    |



conditions of the experiment). The data are not significant. The data from this preliminary series of experiments are not included with the rest, in which larger dosages were employed, as it was not the purpose of the present experiments to determine how the effect, if any, varied with the dosage, but to determine whether there was an effect in the first instance, for which purpose the results got with the maximum tolerable dosage would be preferable.

TABLE 4

| EXP. NO. | TREATMENT      | NO. HRS. | NO. MALES TESTED | NO. F <sub>1</sub> TESTED | LETHALS NO. | %    | MUTATION RATE |
|----------|----------------|----------|------------------|---------------------------|-------------|------|---------------|
| G        | Fast Neutrons  | 16       | 21               | 1882                      | 18          | .96  |               |
| H        | Fast Neutrons  | 40       | 10               | 725                       | 6           | .83  |               |
| K        | Fast Neutrons  | 36       | 18               | 734                       | 8           | 1.09 |               |
| L        | Fast Neutrons  | 36       | 20               | 971                       | 12          | 1.23 |               |
| Total    |                |          | 69               | 4312                      | 44          | 1.02 | 1 in 98       |
| XE       | Gamma-ray Con. | 16       | 17               | 748                       | 3           | .40  |               |
| XG       | Gamma-ray Con. | 16       | 21               | 1554                      | 6           | .39  |               |
| XI       | Gamma-ray Con. | 36       | 5                | 77                        | —           | —    |               |
| XJ       | Gamma-ray Con. | 36       | 11               | 299                       | —           | —    |               |
| XX       | Gamma-ray Con. | 36       | 16               | 958                       | 3           | .31  |               |
| XL       | Gamma-ray Con. | 36       | 19               | 1128                      | 7           | .62  |               |
| Total    |                |          | 89               | 4764                      | 19          | .398 | 1 in 250      |
| E        | Slow Neutrons  | 16       | 11               | 623                       | 1           | .16  |               |
| I        | Slow Neutrons  | 36       | 17               | 568                       | 4           | .70  |               |
| J        | Slow Neutrons  | 36       | 14               | 313                       | —           | —    |               |
| Total    |                |          | 42               | 1504                      | 5           | .332 | 1 in 301      |
| CE       | No Radiation   | —        | 16               | 680                       | 3           | .44  |               |
| CG       | No Radiation   | —        | 9                | 448                       | —           | —    |               |
| CH       | No Radiation   | —        | 7                | 249                       | —           | —    |               |
| CF       | No Radiation   | —        | 6                | 317                       | —           | —    |               |
| CI       | No Radiation   | —        | 10               | 533                       | 4           | .75  |               |
| CJ       | No Radiation   | —        | 16               | 1020                      | —           | —    |               |
| CK       | No Radiation   | —        | 12               | 710                       | 1           | .14  |               |
| CL       | No Radiation   | —        | 21               | 815                       | 4           | .49  |               |
| Total    |                |          | 97               | 4772                      | 12          | .25  | 1 in 398      |

The results of the second two series of experiments, in which larger dosages were used (16 to 40 hour treatment) are shown in tables 2 and 3, respectively, and the combined data in table 4 in more condensed form. A summary of all the results is as follows:

| SERIES           | NO. OF<br>F <sub>1</sub> BRED | NUMBER OF LETHALS |               |                       | MUTATION RATE     |
|------------------|-------------------------------|-------------------|---------------|-----------------------|-------------------|
|                  |                               | TOTAL             | 1 PER<br>MALE | 2 OR MORE<br>PER MALE |                   |
| 1. Fast neutrons | 4312                          | 44                | 18            | 26                    | 0.01021 ± 0.00103 |
| 2. Slow neutrons | 1504                          | 5                 | 5             | 0                     | 0.00332 ± 0.00100 |
| 3. Gamma-rays    | 4764                          | 19                | 19            | 0                     | 0.00399 ± 0.00062 |
| 4. No radiation  | 4772                          | 12                | 12            | 0                     | 0.00252 ± 0.00049 |

The combined results got with the fast neutrons are definitely significant in comparison with the gamma-ray controls. The difference between the frequency rates in 1 and 3 is six times the probable error in 1 and ten times the probable error in 3. The slow neutrons, on the other hand, give no significant result in comparison with the gamma-ray controls, the difference in the two rates being only 0.62 times the probable error in 2 and 1.8 times the probable error in 3. The gamma-rays are having a slight effect. The difference between the frequency rates in 3 and 4 is 2.3 times the probable error in 3 and 3.0 times the probable error in 4.

The number of lethals per male is noteworthy. The total number of males that yielded lethals in the fast neutron-treated series was 25, of which 11 yielded two or more lethals apiece (giving the total of 26 shown in the above summary). This contrasts sharply with the results of the gamma-ray controls and the remaining series, in that there was no tendency for any such grouping of the lethals in them.

Only one inversion was obtained in the present series of experiments. The inversion, located in region I (between  $w^e$  and  $v$ ) was found among the flies treated for 36 hours with fast neutrons (table 3).

#### DISCUSSION

It is not surprising that slow neutrons produced no effect, since these would not as a rule produce dense tracks of ions in matter containing a large proportion of hydrogen, as is true of living tissue. Any hydrogen atoms that absorbed a neutron would give off very short-wave radiation, similar to gamma-rays, and this would as a rule pass out of the tissue without causing any change, if the tissue were very small in volume (although the gamma-rays might sometimes happen to produce ions in the tissue and thus cause mutations). The carbon and nitrogen in living tissues would not be disintegrated by neutrons. Any boron or lithium that was present in the tissues would be broken down with the liberation of alpha particles and protons, and these would produce mutations by means of the dense tracks of ions that they produce. However, there is not much boron or lithium in living tissues ordinarily. The physiological effects produced

in rats (LAWRENCE and LAWRENCE 1936), it will be recalled as well as the retardation of growth in wheat seedlings (ZIRKLE and AEBERSOLD 1936), were due to fast neutrons.

The grouping of the lethals in the fast neutron-treated series calls for special comment. Since adults were treated, the grouping could not be due to "reduplication" of lethals produced in embryonic or germ tract cells. Apparently, the only possible interpretation of these results is that if a neutron produces any "hit" at all, it tends to strike in more than one place. The vast majority of the neutrons would, of course, not be absorbed or deflected and would pass right through the narrow limits of the sperm cells without producing any effects on them. Those that are deflected within the mass of sperm cells would produce a track of ions among the sperm cells (in the path of the recoil protons), and it is to be expected that this track might produce more than one mutation, since the distance between ions is very short (in comparison with the distance between ions produced by gamma-rays).

A large part of the effect of fast neutrons must depend upon the presence of hydrogen in the tissues and be due to the short-range, densely ionizing paths of the recoil protons. Hydrogen, however, is not the only element that can act as a scatterer. Recoil atoms of other elements found in the protoplasm may play a part; especially is this likely if the neutrons are of very high velocity. If there is any direct relation between the biological effectiveness of ionizing particles and the specific ionization of the particles, it is probable that recoil atoms of elements other than hydrogen may affect that process. So we cannot assume that the biological effects of fast neutrons are confined to the effects of recoil-protons. Ionization resulting from collisions of neutrons with other elements may appreciably affect the part of cells which may be especially sensitive to ionization.

#### SUMMARY

Fast neutrons are capable of producing mutations, as indicated by the fact that in the treated series, 44 lethals appeared in 4312  $F_1$  females, derived from 69 eosin males, a mutation rate of 1 in 98, as compared with 19 lethals in 4764  $F_1$  females, derived from 89 eosin males, a mutation rate of 1 in 250, in the gamma-ray controls. The lethal mutation rate of fast neutron-treated flies is therefore 2.6 times that of the corresponding gamma-ray controls. The difference between the frequency rates in the fast neutron series and gamma-ray controls is six times the probable error in the fast neutron series, and ten times the probable error in the gamma-ray controls.

Slow neutrons did not, under the conditions of the experiment, affect the mutation rate. Only 5 lethals appeared in 1504  $F_1$  females (obtained

from 42 eosin males), a rate of 1 in 300.8. The difference between the frequency rates in the slow neutron series and the gamma-ray controls was only 0.62 times the probable error in the slow neutron series, and 1.8 times the probable error in the gamma-ray controls.

There was a tendency for a treated male to produce two or more mutations, if he produced any mutations at all. Since adults were treated, this grouping of the mutations was not due to "reduplication" of mutations produced in the early germ tract, but to separate mutations. This would indicate that there was a tendency for several mutations to occur in each track of ions caused by a neutron (in cases of "hits").

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# THE GENETICS OF SELF-INCOMPATIBILITY IN *OENOTHERA ORGANENSIS*<sup>1</sup>

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Received October 13, 1937

**O***OENOTHERA organensis* is endemic to the Organ Mountains of southern New Mexico where the entire population apparently consists of considerably less than one thousand individuals.<sup>2</sup> The morphology of the seeds indicates a relationship to members of the subgenus *Oenothera* (sometimes called *Onagra*), but the growth habit and ovule placentation resemble types belonging to the subgenus *Raimannia* (the *Euoenothera* of Engler and Prantl). *Oenothera organensis* has proved completely cross-sterile with five species of *Raimannia*. Hybrids with *Oenothera* species are obtained infrequently, and are almost completely sterile, resembling *Oenothera-Raimannia* hybrids. *Oenothera organensis* differs from both these subgenera in being completely self-sterile.

The inheritance of self-incompatibility in *Oe. organensis* proved to be of the *Nicotiana* type, governed by a series of multiple allelomorphs. Interest attaches not so much to the mode of inheritance as to the methods by which genetic analyses can be followed in this material.

## MATERIAL

Seeds of *Oenothera organensis* were collected at Modoc Waterfall, at the mouth of Filmore Canyon on the western slope of the Organ Mountains by Dr. E. W. ERLANSON and Dr. A. E. ARCHER in November, 1928. Plants from these seeds were grown in Pasadena in 1929, 1930 and 1931, and were continued from a single open-pollinated capsule from one of these 1931 plants. The culture grown in 1934 from these 1931 seeds was the first to be tested for self- and cross-incompatibility. Ten plants tested proved to be self-sterile. Nine of these plants were pollinated by a tenth, eight being cross-fertile and one cross-sterile. The first experiments to be reported were made on the progenies of these cross-fertile combinations.

In 1935, Professor P. A. MUNZ collected seeds from plants growing in the type locality, Dripping Springs, in the next canyon south of Filmore Canyon. Professor R. E. CLELAND grew plants from these seeds in Balti-

<sup>1</sup> *Oenothera organensis* Munz n. nom. *Oe. macrosiphon* Woot. & Standl., Contr. U. S. Nat. Herb. 16. 155. 1913, not *Oe. macrosiphon* Lehm. in Hamb. Gartenz. XIV: 439. 1858.

<sup>2</sup> In September, 1937 there were 70 plants in one canyon at Dripping Springs, 42 plants in another canyon at Dripping Springs, less than 20 plants at Modoc Waterfall (June 1937), and about 20 plants in one fork of McAllister Canyon on the north slope of the mountains. Unless there are some unexpected stands of this species in other parts of the mountains, the total population must be less than 500.

more the following year, found that they were self-sterile and made intercrosses between seven of them. Seeds of these crosses were sent me and the progenies were tested at Pasadena in the summer of 1937.

## CROSS-INCOMPATIBILITY IN ONE FAMILY

A culture of 21 plants was grown in the summer of 1936 from seeds of a cross between two members of the 1934 culture of the Modoc strain. Fifteen of these (including all then in flower) were intercrossed in nearly all possible combinations. The first results were extremely complicated: reciprocal crosses reacted alike in some instances and not in others. Following repeated pollinations, seeds were finally obtained in both or neither directions in most reciprocal crosses, and the family could be classified into four intra-sterile, inter-fertile groups. The data obtained are summarized in table 1.

TABLE 1

*Results of cross-pollinations within one family. A set of seeds is indicated by the + sign, the failure of the capsule to set by the - sign.*

| ♀ \ ♂  | TYPE A |     |      |    | TYPE B |    |   | TYPE C |    | TYPE D |
|--------|--------|-----|------|----|--------|----|---|--------|----|--------|
|        | 6      | 8   | 9    | 10 | 1      | 4  | 7 | 18     | 3  | 12     |
| TYPE A | 2      | --  | --   | -  | -      | ++ | + | +++    | -+ | +      |
|        | 5      | --  | -    | -  | -      | +  | + | +      | +  | ++     |
|        | 6      | --  | --   | -  | --     | +  | + | ++     | ++ | ++     |
|        | 8      | --  | ---- | -  | -      | +  | + | ++     | +  | ++     |
|        | 9      | -   | --   | -- | -      | +  | + | ++     | +  | ++     |
|        | 10     | --  | --   | -  | -      | -  | + | +      | +  | ++     |
|        | 20     | -   | --   | -  | -      | +  | + | +      | -- | ++     |
|        | 20     | -   | --   | -  | -      | +  | + | +      | -- | ++     |
| TYPE B | 1      | -+  |      |    |        | -  | - | -      | +  | +      |
|        | 4      | ++  | +    | +  | ++     | -  | - | -      | +  | +      |
|        | 7      | --+ | ++   | +  | +      | -  | - | ---    | -+ | +      |
| TYPE C | 18     | -   |      |    |        |    |   | -      | -- |        |
|        | 3      | ++  | +    | +  | ++     | +  | + | +      | -  | ---    |
|        | 14     | -   | +    |    |        | +  | + |        |    | -      |
|        | 15     | --  | +    | ++ | ++     | -+ | - |        | -  | -      |
| TYPE D | 12     | -+  | ++   | +  | ++     | +  | + | ++     | +  | +      |

## INCOMPATIBILITY TESTS WITHIN THE STYLAR TISSUE

Flowers which had been pollinated 24 hours earlier were removed from the plant, killed, the styles dissected and stained by the method of BUCHHOLZ (1931) for *Datura*. Following compatible cross-pollination, the

pollen tubes had just reached the ovaries (150 to 180 millimeters below the stigma), whereas following self-pollination the pollen tubes remained in the stigma and were mostly less than one millimeter in length.

On flowers removed from the plant and kept in a moist chamber at 25°C, pollen tubes from compatible pollinations grew at nearly a constant rate. Pollen tubes from self-pollinations apparently stopped growing after the first hour or two (fig. 1).

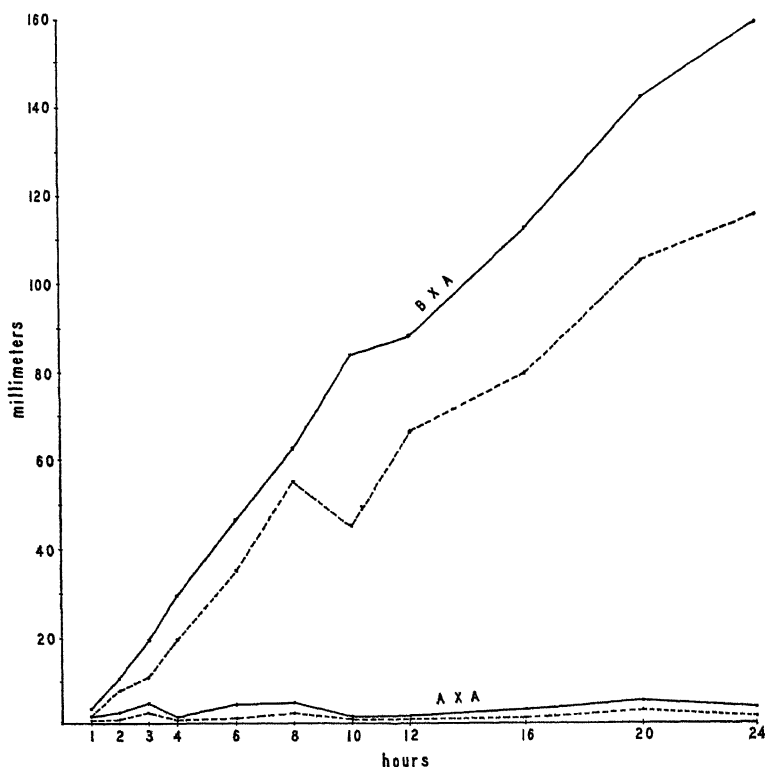


FIGURE 1.—Curves showing the growth of compatible (B×A) and incompatible (A×A) pollen tubes in the styles of flowers removed from the plants. The solid lines show the lengths of the longest pollen tubes, the dotted lines show the mean lengths of all pollen tubes in the styles.

The distinction between compatible and incompatible combinations is clearly evident after four hours, at which time the longest pollen tubes are still within the relatively thick portion of the style (approximately 1 millimeter in diameter) where dissections are readily made. All subsequent tests for cross-incompatibility have been made by this method. In figure 2 the results of tests made in the summer of 1936 are summarized. In these tests the time varied from four to five hours and the temperature from 20° to 27°C. The tests are generally very clear, with the exception of occasional flowers in which incompatible pollen tubes grew longer than is customary,

and in such flowers very few pollen tubes reacted in this way. Consequently, tests made by this method proved much more reliable than those in which the failure of seeds to set was used as a criterion of incompatibility.

The culture which had previously been tested by the set of capsules (table 1) proved to have 8 plants of type A, 5 of type B, 4 of type C and 3 of type D, and there was one plant that failed to flower. Other cultures grown in that year were not tested as completely, but only one culture contained plants belonging to a different incompatibility group. This culture had 8 plants of type C and 3 of type E, with 11 that failed to flower.

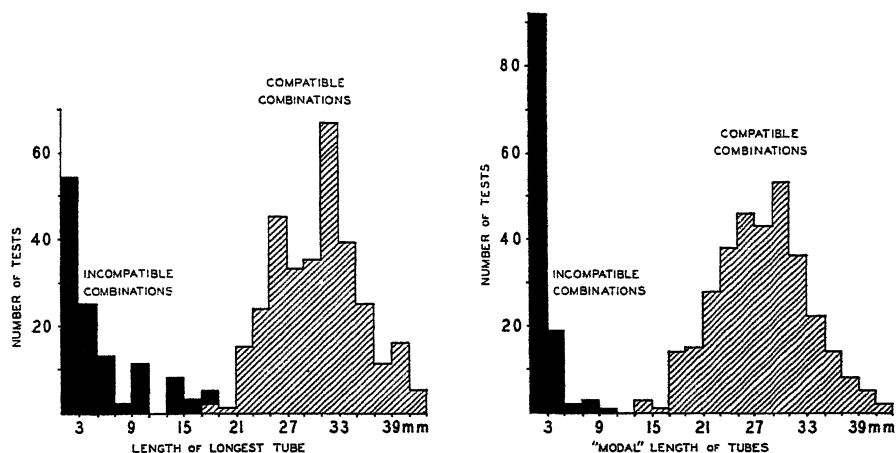


FIGURE 2.—Distribution curves of pollen tubes of compatible and incompatible combinations 4 to 5 hours after pollination. The modal length was determined by inspection, not from measurements of all tubes.

#### PROGENY TESTS

Intercrosses between the different incompatibility groups were grown in the greenhouse in the winter of 1936-37 where their flowering was induced by artificial illumination (long day treatment). The frequencies of the various incompatibility types appearing in these cultures are summarized in table 2, to which have been added data from similar crosses grown in the summer of 1937.

The data indicate that a series of multiple allelomorphs are responsible for self-incompatibility in *Oenothera organensis*, as in *Nicotiana* (EAST and MANGELSDORF 1925) and many other plants. The action of these allelomorphs is such that pollen carrying any particular allelomorph fails to produce normally developing pollen tubes in styles heterozygous (or homozygous) for that allelomorph. In those crosses in which the parents had completely dissimilar allelomorphs, four sterility types are expected



TABLE 2

*Frequencies of different incompatibility types in the progenies of intercrosses between types A, B, C, D and E.*

| CROSS | GENOTYPES OF PARENTS   | TYPE A<br>$S_1S_2$                                    | TYPE B<br>$S_3S_4$                     | TYPE C<br>$S_1S_3$                               | TYPE D<br>$S_2S_4$                               | TYPE E<br>$S_2S_3$                                    | TYPE F<br>$S_1S_4$                               |
|-------|------------------------|---|--|--|--|---|--|
| A×B   | $S_1S_2 \times S_3S_4$ | .....   | .....                                  | $\begin{Bmatrix} 5 \\ 4 \\ 6 \\ 6 \end{Bmatrix}$ | $\begin{Bmatrix} 4 \\ 0 \\ 1 \\ 2 \end{Bmatrix}$ | $\begin{Bmatrix} 3 \\ 0 \\ 3 \\ 9 \end{Bmatrix}$      | $\begin{Bmatrix} 9 \\ 1 \\ 2 \\ 1 \end{Bmatrix}$ |
| B×A   | $S_3S_4 \times S_1S_2$ | .....   | .....                                  | 1  | 1  | 1   | 1  |
| A×C   | $S_1S_2 \times S_1S_3$ | .....   | .....                                  | $\begin{Bmatrix} 2 \\ 4 \\ 10 \end{Bmatrix}$     | .....  | $\begin{Bmatrix} 3 \\ 2 \\ 16 \end{Bmatrix}$          | .....  |
| C×A   | $S_1S_3 \times S_1S_2$ | $\begin{Bmatrix} 1 \\ 2 \\ 7 \\ 9 \\ 5 \end{Bmatrix}$ | .....                                  | .....  | .....  | $\begin{Bmatrix} 1 \\ 1 \\ 3 \\ 9 \\ 4 \end{Bmatrix}$ | .....  |
| A×D   | $S_1S_2 \times S_2S_4$ | .....   | .....                                  | .....  | $\begin{Bmatrix} 3 \\ 0 \end{Bmatrix}$           | .....   | $\begin{Bmatrix} 2 \\ 1 \end{Bmatrix}$           |
| D×A   | $S_2S_4 \times S_1S_2$ | 3   | .....                                  | .....  | .....  | .....   | 0  |
| B×C   | $S_3S_4 \times S_1S_3$ | .....   | .....                                  | $\begin{Bmatrix} 5 \\ 2 \end{Bmatrix}$           | .....  | .....   | $\begin{Bmatrix} 1 \\ 6 \end{Bmatrix}$           |
| C×B   | $S_1S_3 \times S_3S_4$ | .....   | 1                                      | .....  | .....  | .....   | 2  |
| B×D   | $S_3S_4 \times S_2S_4$ | .....   | .....                                  | .....  | 1  | 1   | .....  |
| D×B   | $S_2S_4 \times S_3S_4$ | .....   | 0                                      | .....  | .....  | 1   | .....  |
| C×D   | $S_1S_3 \times S_2S_4$ | $\begin{Bmatrix} 1 \\ 0 \end{Bmatrix}$                | $\begin{Bmatrix} 2 \\ 0 \end{Bmatrix}$ | .....  | .....  | $\begin{Bmatrix} 2 \\ 2 \end{Bmatrix}$                | $\begin{Bmatrix} 0 \\ 0 \end{Bmatrix}$           |
| D×C   | $S_2S_4 \times S_1S_3$ | 2   | 0                                      | .....  | .....  | 1   | 3  |
| E×A   | $S_2S_3 \times S_1S_2$ | 12  | .....                                  | 4  | .....  | .....   | .....  |
| E×B   | $S_2S_3 \times S_3S_4$ | .....   | 7                                      | .....  | 4  | .....   | .....  |
| E×C   | $S_2S_3 \times S_1S_3$ | 10  | .....                                  | 12   | .....  | .....   | .....  |
| E×D   | $S_2S_3 \times S_2S_4$ | .....   | 13                                     | .....  | 7  | .....   | .....  |

among the offspring, none of which represents either parental type. In those crosses in which the parents had one allelomorph in common, only two sterility types are expected among the offspring, and one of these should be identical to that of the male parent.

The allelomorphs responsible for the present types may be determined by the following method:

The cross A×B yields four types, C, D, E and F, hence A and B can have no allelomorph in common. A may be taken as  $S_1S_2$ , B as  $S_3S_4$ .

The cross A×C produces only two types, C and E, hence A and C have one allelomorph in common and this is taken as  $S_1$ .

The cross B×C also produces two types, C and F, and the allelomorph which B and C must have in common is taken as  $S_3$ . Then C has the constitution  $S_1S_3$ .

In the cross A ( $S_1S_2$ ) $\times$ C ( $S_1S_3$ ), only  $S_3$  pollen functions, producing  $S_1S_3$  which is type C and  $S_2S_3$  which must then be type E.

In the cross B ( $S_3S_4$ ) $\times$ C ( $S_1S_3$ ), only  $S_1$  pollen functions, producing  $S_1S_3$  which is type C and  $S_1S_4$  which must then be type F.

Since the crosses A $\times$ D and B $\times$ D each produce but two types including that of the pollen parent, D must have one allelomorph in common with type A and one with type B, and since C and D can have no allelomorph in common (the four types A, B, E and F are produced in intercrosses between them). D must be  $S_2S_4$ .

The remaining crosses listed in table 2 serve as a check on this interpretation. The o's included in this table indicate classes that should have occurred in the particular families but which were not observed.

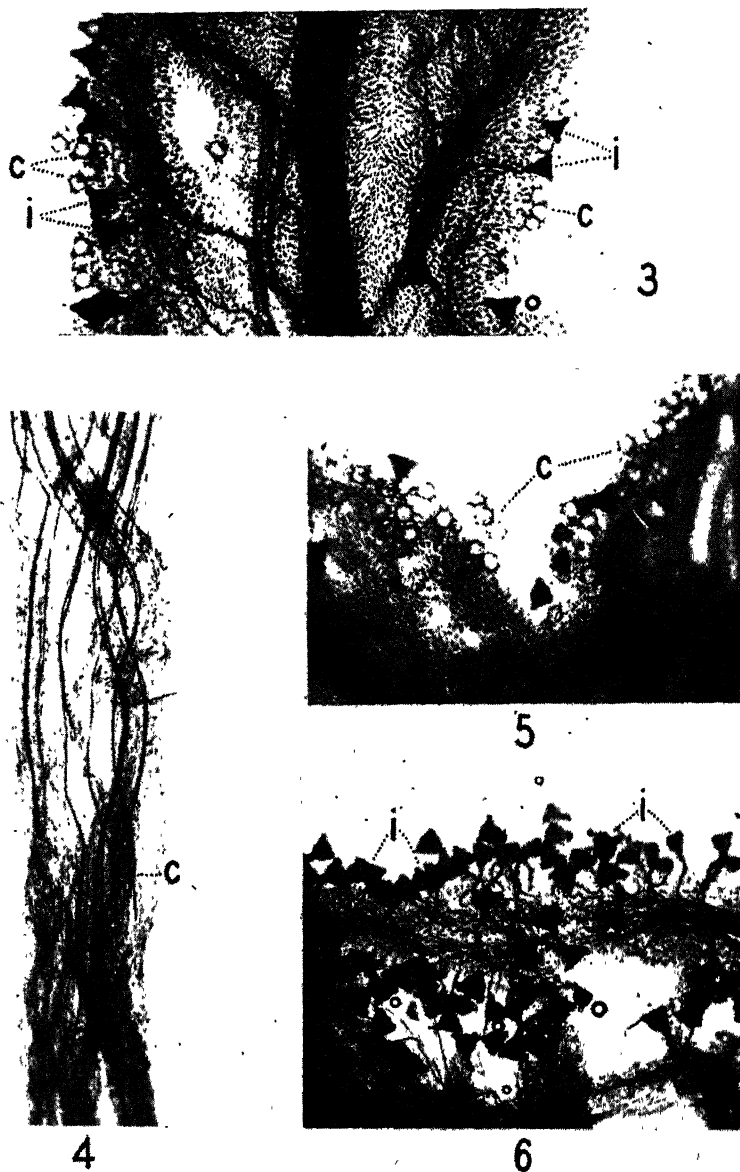
#### TESTS FOR ADDITIONAL ALLELOMORPHS IN THE GAMETOPHYTE GENERATION

An examination of stigmas prepared four hours after pollination showed that it is possible to distinguish between the two sorts of pollen in crosses between types having an allelomorph in common, for example  $S_1S_2\times S_1S_3$ . Figure 3 is a photomicrograph of a stigma following this type of pollination. The incompatible pollen (carrying the allelomorph also present in the stylar tissue) produces very short tubes and the pollen grains retain their contents. The compatible pollen produces very long tubes (fig. 4) and the pollen grains and the upper portions of the tubes are empty and fail to stain. Figure 5 is a photograph of a completely compatible combination in which all of the germinating pollen has produced long tubes, the pollen grains becoming empty. Figure 6 is a photograph of a completely incompatible combination, equivalent to self-pollination, in which all of the germinating pollen produces short tubes and in which there are no empty pollen grains.

By using the presence of two types of pollen, that with long tubes and that with short, to indicate the presence of an allelomorph common to the two parents, it is possible to determine the genetic constitution of any particular plant without progeny tests. This method was used in analyzing the crosses obtained from Professor CLELAND (the Dripping Springs Strain). The data from these tests are presented in figures 7 and 8. Figure 7 is a table showing the behavior of pollen in intercrosses between the various incompatibility types occurring. Figure 8 is a table showing the incompatibility groups expected and the observed frequencies of plants belonging to these groups in the different crosses.

The details of the analysis follow:

Type O has one allelomorph in common with types C ( $S_1S_3$ ) and E ( $S_2S_3$ ) none with type A ( $S_1S_2$ ), hence O has allelomorph  $S_3$ . Type O has no



FIGURES 3 to 6.—Photomicrographs of portions of prepared stigmas or styles four hours after pollination (magnification 22 $\times$ ). Figure 3, a partially compatible combination ( $S_7S_8 \times S_6S_7$ ); c, compatible ( $S_6$ ) pollen; i, incompatible ( $S_7$ ) pollen. Figure 4, portion of style 20 mm below the stigma from the same preparation as Figure 3 to show ends of compatible pollen-tubes. Figure 5, completely compatible combination ( $S_8S_7 \times S_6S_8$ ); the few stained pollen-grains failed to germinate. Figure 6, incompatible combination ( $S_6S_8 \times S_6S_8$ ) equivalent to self-pollination.

allelomorph in common with type F ( $S_1S_4$ ), hence the second allelomorph carried by O is different from all previously determined and is taken as  $S_5$ . Type O is  $S_3S_5$ .

Type G has one allelomorph in common with type O ( $S_3S_5$ ), none with

| ♂ | A              | C             | E             | F             | D             | B             | O             | P             | G             | Q             | J             | H             | M             | S             | V             | T             | L             | N             | K             | Z              | Γ              | Φ              |
|---|----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|----------------|----------------|----------------|
| ♀ | $\frac{1}{2}$  | $\frac{1}{3}$ | $\frac{2}{3}$ | $\frac{1}{4}$ | $\frac{2}{4}$ | $\frac{3}{4}$ | $\frac{3}{5}$ | $\frac{3}{6}$ | $\frac{5}{6}$ | $\frac{3}{7}$ | $\frac{5}{7}$ | $\frac{6}{7}$ | $\frac{5}{8}$ | $\frac{6}{8}$ | $\frac{7}{8}$ | $\frac{5}{9}$ | $\frac{6}{9}$ | $\frac{7}{9}$ | $\frac{8}{9}$ | $\frac{6}{10}$ | $\frac{7}{10}$ | $\frac{7}{11}$ |
| C | $\frac{1}{3}$  | -             | $\frac{1}{2}$ | $\frac{1}{2}$ | +             |               |               |               |               |               |               |               |               |               |               |               |               |               |               |                |                |                |
| E | $\frac{2}{3}$  |               | $\frac{1}{2}$ | -             | +             | $\frac{1}{2}$ |               |               |               |               |               |               |               |               |               |               |               |               |               |                |                |                |
| F | $\frac{1}{4}$  |               | $\frac{1}{2}$ | +             | -             | $\frac{1}{2}$ |               |               |               |               |               |               |               |               |               |               |               |               |               |                |                |                |
| D | $\frac{2}{4}$  |               | +             | $\frac{1}{2}$ | $\frac{1}{2}$ | -             |               |               |               |               |               |               |               |               |               |               |               |               |               |                |                |                |
| B | $\frac{3}{4}$  | +             | $\frac{1}{2}$ |               |               | $\frac{1}{2}$ | -             |               |               |               |               |               |               |               |               |               |               |               |               |                |                |                |
| O | $\frac{3}{5}$  | +             | $\frac{1}{2}$ | $\frac{1}{2}$ | +             | +             | $\frac{1}{2}$ | -             | $\frac{1}{2}$ | $\frac{1}{2}$ | $\frac{1}{2}$ | $\frac{1}{2}$ | +             | $\frac{1}{2}$ | +             | +             |               | +             | +             | +              | +              | +              |
| P | $\frac{3}{6}$  |               | $\frac{1}{2}$ |               |               |               |               | $\frac{1}{2}$ | -             | $\frac{1}{2}$ |               | +             | $\frac{1}{2}$ |               |               |               |               |               |               |                |                |                |
| G | $\frac{5}{6}$  | +             | +             | +             | +             | +             | $\frac{1}{2}$ |               | -             |               |               | $\frac{1}{2}$ |               |               |               |               |               |               |               |                |                |                |
| Q | $\frac{3}{7}$  | +             | $\frac{1}{2}$ |               |               |               | $\frac{1}{2}$ |               | +             | -             | $\frac{1}{2}$ | $\frac{1}{2}$ | +             |               |               |               |               | $\frac{1}{2}$ |               |                |                |                |
| J | $\frac{5}{7}$  | +             | +             | +             | +             | +             | $\frac{1}{2}$ |               | $\frac{1}{2}$ | $\frac{1}{2}$ | -             | $\frac{1}{2}$ | $\frac{1}{2}$ | +             | $\frac{1}{2}$ | $\frac{1}{2}$ | +             | $\frac{1}{2}$ | +             | +              | $\frac{1}{2}$  | $\frac{1}{2}$  |
| H | $\frac{6}{7}$  | +             | +             | +             | +             | +             | +             |               | $\frac{1}{2}$ | $\frac{1}{2}$ | $\frac{1}{2}$ | -             | +             | $\frac{1}{2}$ | $\frac{1}{2}$ | +             | $\frac{1}{2}$ | $\frac{1}{2}$ | +             | $\frac{1}{2}$  | $\frac{1}{2}$  | $\frac{1}{2}$  |
| M | $\frac{5}{8}$  |               |               |               |               |               |               |               | $\frac{1}{2}$ |               | $\frac{1}{2}$ | +             | -             | $\frac{1}{2}$ | $\frac{1}{2}$ |               |               |               | $\frac{1}{2}$ |                |                | +              |
| S | $\frac{6}{8}$  |               |               |               |               |               | +             |               | $\frac{1}{2}$ |               | +             | $\frac{1}{2}$ | $\frac{1}{2}$ | -             | $\frac{1}{2}$ |               | $\frac{1}{2}$ | +             | $\frac{1}{2}$ |                |                |                |
| V | $\frac{7}{8}$  |               |               |               |               |               | +             | +             | $\frac{1}{2}$ | $\frac{1}{2}$ | $\frac{1}{2}$ | $\frac{1}{2}$ | $\frac{1}{2}$ | $\frac{1}{2}$ | -             |               | +             | $\frac{1}{2}$ | $\frac{1}{2}$ | +              | $\frac{1}{2}$  | $\frac{1}{2}$  |
| W | $\frac{3}{9}$  |               |               |               |               |               |               |               |               | $\frac{1}{2}$ |               | +             |               |               |               |               | $\frac{1}{2}$ |               |               |                |                |                |
| T | $\frac{5}{9}$  |               |               |               |               |               | $\frac{1}{2}$ | +             | $\frac{1}{2}$ |               | $\frac{1}{2}$ | +             |               |               |               |               | $\frac{1}{2}$ |               | $\frac{1}{2}$ |                |                |                |
| L | $\frac{6}{9}$  | +             |               |               |               | +             |               |               | $\frac{1}{2}$ | +             | +             | $\frac{1}{2}$ | +             | +             | +             |               | -             | $\frac{1}{2}$ | $\frac{1}{2}$ |                | +              |                |
| N | $\frac{7}{9}$  | +             | +             | +             | +             | +             | +             |               | +             | $\frac{1}{2}$ | $\frac{1}{2}$ | $\frac{1}{2}$ | +             | +             | $\frac{1}{2}$ |               | $\frac{1}{2}$ | -             | $\frac{1}{2}$ | +              |                | $\frac{1}{2}$  |
| K | $\frac{8}{9}$  |               |               | +             | +             |               |               |               | +             |               | +             | +             |               |               | $\frac{1}{2}$ |               |               |               | -             |                |                | +              |
| X | $\frac{3}{10}$ |               |               |               |               |               |               |               |               | $\frac{1}{2}$ |               | +             |               |               |               |               |               |               |               |                |                |                |
| Z | $\frac{6}{10}$ | +             | +             | +             | +             | +             |               |               | $\frac{1}{2}$ |               | +             | $\frac{1}{2}$ | +             | $\frac{1}{2}$ | +             |               | $\frac{1}{2}$ | +             | +             | -              | $\frac{1}{2}$  | +              |
| Γ | $\frac{7}{10}$ | +             | +             | +             |               | +             | +             |               | +             | +             |               |               | +             |               | $\frac{1}{2}$ |               |               | $\frac{1}{2}$ | +             | $\frac{1}{2}$  |                | $\frac{1}{2}$  |
| Θ | $\frac{3}{11}$ |               |               |               |               |               |               |               |               | $\frac{1}{2}$ |               | +             |               |               |               |               |               |               |               |                |                |                |
| Φ | $\frac{7}{11}$ | +             | +             |               |               | +             | +             |               | +             |               | $\frac{1}{2}$ | $\frac{1}{2}$ |               |               | $\frac{1}{2}$ |               |               | $\frac{1}{2}$ |               |                |                |                |

FIGURE 7.—Behavior of pollen-tubes in crosses between the different incompatibility types. The + symbol indicates a completely compatible combination, - a completely incompatible combination and  $\frac{1}{2}$  a combination with one allelomorph common to both parents. The letters in the headings refer to the incompatibility types, the fractions under or beside them refer to the allelomorphs of  $S$  carried by each type, for example,  $\frac{3}{5}$  represents  $S_3S_5$ , etc.

types A to F ( $S_1$  to  $S_4$ ), hence G has  $S_5$  and no other previously determined allelomorph. Type G is  $S_5S_6$ .

Type H has one allelomorph in common with type G ( $S_5S_6$ ), none with type O ( $S_3S_5$ ), hence H carries  $S_6$  and no other previously identified allelo-

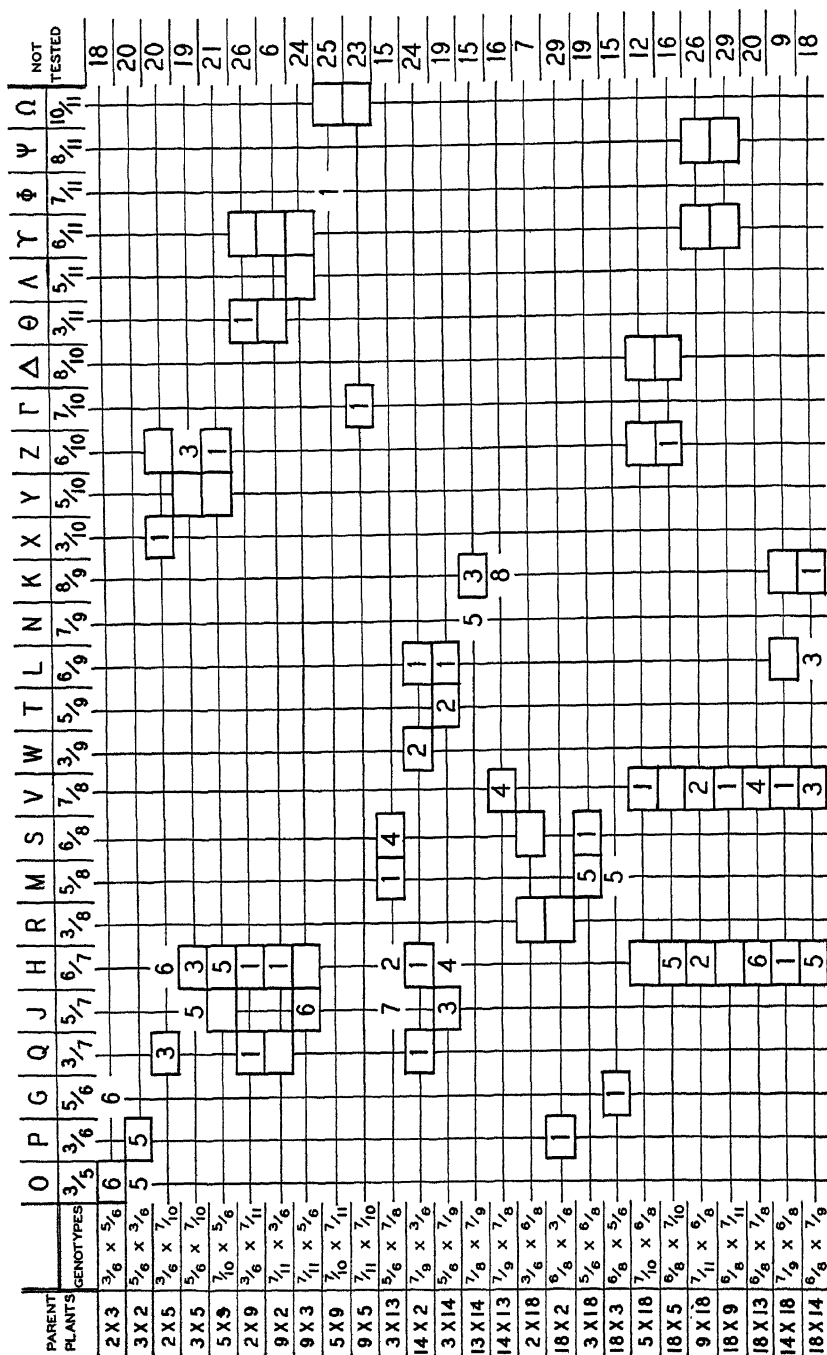


FIGURE 8.—Frequencies of different sterility types in different intercrosses. The heavy-lined squares in the table indicate predicted classes (see text).

morph, as shown by crosses with types A to F ( $S_1$  to  $S_4$ ). Type H is  $S_6S_7$ .

Type J has one allelomorph in common with each of types O ( $S_3S_5$ ), G ( $S_5S_6$ ) and H ( $S_6S_7$ ). Type J does not have  $S_3$  as it has no allelomorph in common with type C ( $S_1S_3$ ). Hence J has  $S_5$  in common with types O and G,  $S_7$  with type H. Type J is  $S_5S_7$ .

Type K has no allelomorph in common with types E ( $S_2S_3$ ), F ( $S_1S_4$ ), G ( $S_5S_6$ ) or J ( $S_5S_7$ ). Type K is  $S_8S_9$ .

Type L has one allelomorph in common with type G ( $S_5S_6$ ), none with type J ( $S_5S_7$ ), hence L has  $S_6$ . Type L has one allelomorph in common with type K which can be either  $S_8$  or  $S_9$ . Type L is  $S_6S_9$ .

Type M has one allelomorph in common with type J ( $S_5S_7$ ), none with type H ( $S_6S_7$ ), hence M has  $S_5$ . It has one allelomorph in common with type K ( $S_8S_9$ ), none with type L ( $S_6S_9$ ), hence M has  $S_8$ . Type M is  $S_5S_8$ .

Type N has one allelomorph in common with types J ( $S_5S_7$ ) and L ( $S_6S_9$ ) none with type G ( $S_5S_6$ ), therefore N has  $S_7S_9$ .

Type Z has one allelomorph in common with type G ( $S_5S_6$ ), none with type J ( $S_5S_7$ ), hence type Z has  $S_6$ . Type Z has no allelomorph in common with types A to F ( $S_1$  to  $S_4$ ) nor with type K ( $S_8S_9$ ). Type Z is  $S_6S_{10}$ .

Type  $\Phi$  has one allelomorph in common with type J ( $S_5S_7$ ), none with type G ( $S_5S_6$ ), hence  $\Phi$  has allelomorph  $S_7$ . Type  $\Phi$  has no allelomorphs in common with types A ( $S_1S_2$ ), B ( $S_3S_4$ ), K ( $S_8S_9$ ) or Z ( $S_6S_{10}$ ). Type  $\Phi$  is  $S_7S_{11}$ .

After these types had been identified, the genetic constitutions of the parents were deduced. It was then possible to predict the types that should occur in all intercrosses. The method of determining the constitutions of the parents is as follows:

#### *Parent no. 3.*

$\#2 \times \#3$  produced type G,  $\#3$  has  $S_5$  or  $S_6$  or both.

$\#3 \times \#2$  produced type O,  $\#3$  has  $S_3$  or  $S_5$ , hence  $\#3$  is one of  $S_3S_6$ ,  $S_5S_6$ ,  $S_5S_x$ .

$\#3 \times \#13$  produced type H,  $\#3$  has  $S_6$  or  $S_7$ , hence  $S_3S_6$ ,  $S_5S_6$  or  $S_5S_7$ .

$\#3 \times \#13$  produced type J,  $\#3$  has  $S_5$  or  $S_7$  not both, hence parent no. 3 is  $S_5S_6$  or type G.

#### *Parent no. 14.*

$\#3 \times \#14$  produced type H ( $S_6S_7$ ) and since  $S_6$  came from  $\#3$ ,  $\#14$  has allelomorph  $S_7$ .

$\#3 \times \#13$  produced type H, hence  $\#13$  similarly has  $S_7$ .

$\#13 \times \#14$  produced type N ( $S_7S_9$ ) and since  $\#13$  and  $\#14$  both have  $S_7$ , the pollen parent must be type N. Parent no. 14 is  $S_7S_9$  or type N.

Parent no. 13.

#13 has been shown above to have  $S_7$ .

#14 × #13 produced type K ( $S_8S_9$ ) and since  $S_9$  is from #14, parent #13 is  $S_7S_8$ , called type V.

Parent no. 5.

#3 × #5 produced types J ( $S_6S_7$ ) and Z ( $S_6S_{10}$ ), and since allelomorphs  $S_6$  and  $S_6$  were from #3, parent no. 5 is  $S_7S_{10}$  called type Γ.

Parent no. 2.

#3 × #2 produced type O ( $S_3S_5$ ) and since  $S_5$  is from #3, #2 must have  $S_3$ .

#2 × #5 produced type H ( $S_6S_7$ ) with  $S_7$  from #5, hence parent no. 2 is  $S_3S_6$  called type P.

Parent no. 9.

#9 × #2 produced type H ( $S_6S_7$ ) with  $S_6$  from #2,

#5 × #9 produced type Φ ( $S_7S_{11}$ ) with  $S_7$  from #9, parent no. 9 is  $S_7S_{11}$  or type Φ.

Parent no. 18.

#18 × #3 produced type M ( $S_5S_8$ ) with  $S_5$  from #3,

#18 × #14 produced type L ( $S_6S_9$ ) with  $S_9$  from #14, parent no. 18 is  $S_6S_8$  called type S.

In figure 8, the heavy-lined squares indicate the additional classes expected in the various crosses. The numbers enclosed in these squares represent the observed frequencies of these classes. Unfortunately a fairly high proportion of the plants failed to flower during the summer so that the tests are necessarily incomplete. The data are sufficient, however, to serve as a reasonably good check on the above determinations.

#### CHROMOSOME CONFIGURATIONS

The culture of *Oenothera organensis* grown in 1931 directly from seeds collected at Modoc Waterfall contained plants with different chromosome configurations. The female parent of the 1934 culture had seven chromosome pairs. At least one other plant had a ring of six chromosomes and four pairs. Plants of the 1934 culture were not examined cytologically, but both configurations appeared in the 1936 culture derived from it. The configurations observed in the 1936 culture discussed earlier were:

|                      |   |                         |
|----------------------|---|-------------------------|
| Type A ( $S_1S_2$ )— | { | 6 plants with 7 pairs   |
|                      | 1 | plant with a ring of 4  |
| Type C ( $S_1S_3$ )— |   | 3 plants with 7 pairs   |
| Type D ( $S_2S_4$ )— |   | 3 plants with ring of 6 |
| Type B ( $S_3S_4$ )— |   | 4 plants with ring of 6 |

The ring of 6 appeared whenever  $S_4$  was associated with either  $S_2$  or  $S_3$ . With the exception of the one plant with a ring of four chromosomes, 7 pairs resulted from associations of  $S_1$  with  $S_2$  and of  $S_1$  with  $S_3$ . The further crosses made to test the association of the ring of 6 with  $S_4$  are reported in table 3. While the tests are not extensive, all of the plants carrying  $S_4$  (a total of 8) had rings of 6 chromosomes, whereas those with combinations of other allelomorphs (a total of 6) had 7 pairs of chromosomes.

TABLE 3

| PARENTS  | PROGENY                   |
|--|---------------------------|
| $S_1S_2$ (7 pairs) $\times$ $S_2S_4$ (ring 6) . . . . .  | 1 $S_2S_4$ with ring of 6 |
|  | 1 $S_1S_3$ with 7 pairs   |
| $S_1S_2$ (7 pairs) $\times$ $S_3S_4$ (ring 6) . . . . .  | 3 $S_1S_4$ with ring of 6 |
|  | 1 $S_2S_3$ with 7 pairs   |
|  | 3 $S_2S_4$ with ring of 6 |
| $S_1S_3$ (7 pairs) $\times$ $S_2S_4$ (ring 6) . . . . .  | 1 $S_1S_2$ with 7 pairs   |
| $S_2S_4$ (ring 6) $\times$ $S_1S_3$ (7 pairs) . . . . .  | 1 $S_1S_2$ with 7 pairs   |
| $S_3S_4$ (ring 6) $\times$ $S_2S_4$ (ring 6) . . . . .   | 1 $S_2S_4$ with ring of 6 |
| $S_1S_2$ (7 pairs) $\times$ $S_1S_3$ (7 pairs) . . . . . | 1 $S_2S_3$ with 7 pairs   |

The exceptional type A plant with a ring of 4 chromosomes ( $S_1S_2$ ) was pollinated by a C-type plant ( $S_1S_3$ ) with 7 pairs. Among the progeny, one  $S_1S_3$  plant had 7 pairs, one  $S_2S_3$  plant had a ring of 4, and three plants of the latter type had 7 pairs of chromosomes. These data, while extremely meagre, indicate that the  $S$  locus is independent of the ring of 4 chromosomes.

Both the ring of six chromosomes and the ring of four are invariably associated with the nucleolus. That the nucleolar chromosome does not contain the  $S$  locus is shown by the inheritance of the ring of four.

#### THE INHERITANCE OF MALE-STERILE

The 1936 culture previously discussed also segregated for male-sterile. In the male-sterile plants, meiosis proceeds normally, but the pollen grains produced are small and deficient in starch content and the anthers fail to dehisce.

Of the 20 plants in the 1936 culture, 5 were male-sterile. One of these belonged to type C ( $S_1S_3$ ), four to type A ( $S_1S_2$ ). Intercrosses between normal sibs showed that male-sterile is a simple recessive and that several of the normal plants were heterozygous for the gene ( $ms$ ) involved. A B-type plant ( $S_3S_4$ ) with a ring of 6 chromosomes and heterozygous for  $ms$  was backcrossed to an A-type ( $S_1S_2$ ) male-sterile. Of the offspring carrying  $S_3$ , 14 were normal and 6 male-sterile; of the progeny carrying  $S_4$ , 2 were normal and 1 male-sterile. These results indicate that  $ms$  is independent of the  $S$  locus and of the ring of 6 chromosomes associated with  $S_4$ .



## SUMMARY AND CONCLUSIONS

Self-incompatibility in *Oenothera organensis* is governed by a series of multiple allelomorphs in the same manner as in *Nicotiana* (EAST and MANGELSDORF, 1925). Pollen carrying any particular allelomorph fails to produce normally developing pollen-tubes in any style also carrying that allelomorph. The scheme was proved to hold for *Oe. organensis* first by progeny tests and secondly by tests of pollen-tube development in the tissues of the style and stigma.

The failure of seed production proved to be an unreliable criterion for determining cross-incompatibility, but direct tests of pollen tube development within the stigma proved entirely satisfactory. These results suggest that certain of the complications observed by SIRKS (1926) in *Verbascum* and by SEARS (1937) in *Brassica* may have been due to the inadequacy of the seed-set test for determining cross-compatibility.

The examination of pollen tube development directly within the stigma has a further advantage in that progeny tests are rendered unnecessary in establishing genetic constitutions.

In the studies to date, eleven allelomorphs have been established. Three of these were found only in material from Modoc Waterfall, seven only in material from Dripping Springs. Only one allelomorph occurred in both samples. An extensive test of the distribution of allelomorphs in the native populations is now under way.

Other characters studied in *Oenothera organensis* were chromosome configurations and male-sterility. Translocations involving three chromosomes are associated with allelomorph  $S_4$  so that all combinations between  $S_4$  and  $S_1$ ,  $S_2$  and  $S_3$  have a ring of six chromosomes. All possible combinations between  $S_1$ ,  $S_2$  and  $S_3$  have normally pairing chromosomes. A ring of four chromosomes appearing in a single plant proved to be independent of the  $S$  locus. Since both the ring of six and the ring of four are invariably associated with the nucleolus,  $S$  cannot be carried by the nucleolar chromosome. Male-sterile ( $ms$ ) proved to be a simple recessive independent of the  $S$  locus and of the ring of six.

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# SEX DETERMINATION IN A "BISEXUAL" STRAIN OF *SCIARA COPROPHILA*<sup>1</sup>

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Received November 1, 1937

## INTRODUCTION

THE present investigation is concerned with a study of sex determination in a strain giving bisexual progenies which was derived from a species of fungus-gnat (*Sciara coprophila*) that typically gives only unisexual progenies. Previous studies have shown that in the species *coprophila* the sex of the individual fly is typically dependent upon the type of elimination of entire chromosomes from nuclei in the developing embryo, and that the type of elimination apparently depends upon the zygotic chromosome complex of the female parent. Hence, each female gives a unisexual progeny. In the bisexual strain of this species, a single female gives both sons and daughters, indicating that not only is sex dependent upon the zygotic chromosome constitution of the mother, but that some other factor enters in as well. Occasional progenies in the bisexual line were unisexual (male). This point will be discussed later. It seemed probable that a thorough investigation of this case might throw light on chromosome behavior and the sex problem in *Sciara*, and also on the general problems of sex determination.

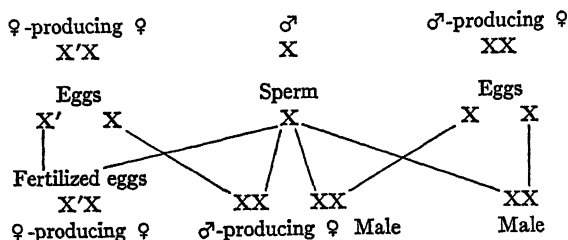
METZ has used the terms monogenic and digenic to designate the two types of reproduction found in species of the genus *Sciara*. Females of the former type produce unisexual progenies, and those of the latter type, bisexual. The use of the terms is carried still further here to designate the type of individual female, that is, a producer of a unisexual progeny is called a monogenic female, and one producing a bisexual progeny, digenic.

The earlier genetic and cytological studies on *Sciara* have been carried out in recent years by METZ and his associates. They have shown that genetically a female of a monogenic strain may have either of two chromosome constitutions. Her sex chromosome constitution may be  $X'X$  or  $XX$ . Flies with the former sex chromosome constitution are female-producing females, giving the two kinds of daughters in about equal numbers, while flies with the  $XX$  chromosome constitution are male-producing females. The evidence (METZ and MOSES 1928; METZ and SMITH 1931; METZ 1931a; METZ and ULLIAN 1929; METZ and SCHMUCK 1929a, 1929b) in-

<sup>1</sup> A dissertation submitted to the Board of University Studies of The Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy in the Department of Zoology, 1934.

dicates that all sperms are alike. Hence the sex of the progeny as a whole is dependent upon the zygotic chromosome constitution of the mother. In table 1 the mode of sex ratio determination is represented.

TABLE 1  
Scheme of sex ratio determination in *Sciara*.



The sex of the individual fly depends finally upon the type of chromosome elimination which takes place during the early cleavage divisions of the developing embryo. The chromosome complex of the fertilized egg, whether it is to develop into male or female, is a small pair of V's, two pairs of rods, and a third rod of which there are three homologous members. Two of these three rods were brought into the fertilized egg by the sperm and are known to be sister halves of the maternally derived X in the male producing the sperm. In developing eggs from  $X'X$  females one of these sister halves of the X chromosome brought in by the sperm is eliminated (seventh or eighth cleavage division) from the soma cells and retained at least for a time by the nuclei making up the primordial germ cells. This leaves the soma with a pair of sex chromosomes. In developing eggs from  $XX$  mothers both sister halves of the sex chromosome from the sperm are regularly eliminated from the soma cells, leaving the soma XO in constitution. Eggs after such elimination develop into males.

In addition to the three pairs of autosomes and the X chromosomes there is a variable number, often a pair (but sometimes only one and sometimes three) of large V-shaped chromosomes which are carried by the germ cells. Since they are limited to the germ line, METZ has termed them the "limited" chromosomes. These are regularly eliminated from the soma. Apparently they play no significant part in sex determination and will, therefore, be disregarded.

Occasionally exceptional females appear in a male progeny and likewise exceptional males may appear in a female progeny. Under ordinary conditions such an exceptional female in a male progeny would have only the usual type of X and would certainly not carry the  $X'$  factor. The bisexual line arose from one of these exceptional females.

METZ (laboratory notes) found that digenic females gave three classes of sons—suggesting the presence of three X chromosomes. Cytological

observations, however, revealed only two X chromosomes in ovarian cells. Since at that time nothing was known of chromosome elimination, the latter evidence was considered as conclusive indication that the digenic females were not XXX.

Dr. HELEN MONOSMITH PEARSON (unpublished data) made a study of this strain and suggested that each digenic female is a mosaic, from fertilization by two sperms, which could thus bring in two different X chromosomes. Later discovery of chromosome elimination put a new light on the subject and led to the present investigation.

#### MATERIALS AND METHODS

The material for the present investigation was a bisexual line obtained from Dr. C. W. METZ. Mutant stocks with which the flies were tested genetically were furnished from the same laboratory.

The flies were reared in glass vials ten cm in diameter. The cultures were kept at about 24°C. Evaporation from pans of water set in the chamber supplied sufficient moisture. The culture methods are those used by Dr. METZ and his colleagues in their work with *Sciara* (SMITH-STOCKING 1936).

#### *Genetic methods*

The results from Dr. METZ's laboratory which were at hand when this investigation was started in the spring of 1932 showed clearly that the sex chromosome was involved in the mechanism effecting the digenic reproduction. The results of the crosses of digenic females with truncate males had shown that the chromosome carrying the gene for truncate was not responsible for the bisexuality.

To test the other two linkage groups virgin females of the bisexual line were mated to males showing mutant characters, the genes for which are carried in one or the other of these two remaining chromosomes. Experiments were carried out with each of the two remaining linkage groups so that each of the two chromosomes was replaced in the bisexual strain by chromosomes from a monogenic line.

The sex chromosomes in the digenic females were replaced by X chromosomes carrying recessive mutant genes. If any one of these chromosomes was in any way responsible for the bisexuality of the line, it would not be possible except by intervention of crossing over to substitute completely chromosomes from the unisexual strains and to have the bisexuality continued. More details in reference to these tests will be brought out in the results.

#### *Cytological methods*

The cytological studies were made on whole eggs mounted in balsam. The technique used was developed in this laboratory in connection with the *Sciara* work (SCHMUCK and METZ 1931).

In order to get eggs in the desired stages of development, a special method of inducing flies to lay their eggs at a desired time was developed. Mated females were put into small glass vials which were one-third full of solidified agar-agar solution. After the agar-agar had shrunken away from the sides of the vial, the female, now two or three days old, was induced to crawl in between the agar-agar and the side of the glass vial, while the former was held back with a dissecting needle. The needle was then removed and the pressure of the agar-agar block on the thorax of the fly induced her to lay the eggs promptly. With this controlled method it was easy to get an abundance of eggs in any stage of development. (The eggs in *Sciara* are fertilized at the time of laying.) Eggs were fixed in Carnoy's 1:1:1 mixture. These were stained with fuchsin sulfurous acid (Feulgen's "Nuclealfärbung"). The staining technique as given by LEE (Ninth edition, page 437) was followed. The eggs were partially hydrolized in 1N HCl at 60°C for ten minutes. They were washed then in SO<sub>2</sub> water for three to five minutes, dehydrated, cleared with xylol, and mounted in balsam. It was necessary to leave the stained eggs in 80 percent alcohol overnight in order to remove the stain from the cytoplasm. After fixation and washing, the eggs were packed into *Drosophila* pupa skins to be carried through the various fluids. They were removed from the pupa skins in the balsam on the slide. The nuclei of the maturation divisions lie near the surface of the egg. By rolling the egg, effected by pushing the cover slip with a dissecting needle, the chromosome group could be viewed from various angles.

#### EXPERIMENTAL RESULTS AND DISCUSSION

As mentioned previously the autosome carrying the mutant gene truncate was shown by METZ and PEARSON not to play any part in the mechanism affecting bisexuality in the bisexual strain. Tests were made to ascertain whether or not either of the other two autosomes was responsible for the bisexuality of the line. The dominant gene Blister is carried in one of these autosomes and Curly, also dominant, in the other.

Virgin females from the bisexual line were out-bred to males from a monogenic line carrying Blister. All F<sub>1</sub> flies showed, of course, the mutant character. When such F<sub>1</sub> mutant females were out-bred to wild type males from a monogenic line, bisexual progenies were obtained showing wild type and Blister flies. In these mutant flies in the second generation both the chromosomes carrying Blister and its homologue were derived from monogenic lines. The digenic reproduction was retained and continued through at least two more generations at which time the lines were discontinued. Similar experiments with Curly showed conclusively that the chromosome carrying this gene could be entirely replaced with monogenic line chromosomes without interfering with the digenic reproduction.

From these results it may be concluded that only the sex chromosome need be taken into consideration.

*Genetic evidence for trisomic germ line in digenic females*

Evidence from genetic experiments shows that three X chromosomes are carried in the germ line of digenic females in the bisexual strain. Three recessive sex-linked mutant stocks were used in these experiments, swollen, round and miniature. Virgin females from the bisexual line were bred to males of one of the mutant stocks. F<sub>1</sub> virgin females were bred to males of the second mutant stock, and similarly F<sub>2</sub> virgin females were out-bred to males of the third mutant stock.

Wild type females in third generation progenies giving three classes of males (wild type, round, and swollen) were bred to swollen males. These swollen males came out of the experiments (male progenies), hence were not directly from a monogenic stock. Most of the progenies from these females again showed three classes of males, representing three possible combinations of three classes. They were wild type, round, miniature; wild type, swollen, miniature; and round, swollen, miniature. These results show conclusively that three X chromosomes were carried in the germ lines of the digenic mothers. The bisexual progenies showing these results are given in table 2.

*Cytological evidence for the trisomic germ line in digenic females*

The best picture of the chromosomes is found in the early part of the second maturation division. At this time the first polar body chromosomes are intact and the number of chromosomes in the germ line of the female can be ascertained.

Since it is seen from the genetic experiments that three sex chromosomes are being carried in the germ line of the digenic females, and since the evidence indicates that the sex chromosome in *coprophila* is one of the rods, an extra rod was expected in these eggs. Many eggs were examined and the extra rod was found. It will be remembered that the diploid number of chromosomes in the monogenic female is eight. In addition to the "limited" chromosomes there are one pair of small V's and three pairs of rods. Figure 1a shows a drawing of the chromosomes in the second oocyte, and figure



FIGURE 1.—Chromosomes in second oocyte (a) and in first polar body (b) in one egg from a digenic female. Only the upper group of split chromosomes is represented. Feulgen preparation.

1b, those in the first polar body in a single egg from a digenic female. These are, of course, haploid groups. The chromosomes are already split

TABLE 2

*Bisexual progenies showing three classes of males, representing three X chromosomes in the germ line of mothers. Progenies are grouped according to classes of males.*

(a) Wild type (+), round (r), and miniature (m) males.

| ♀   |   | ♂  |     |     |     |
|-----|---|----|-----|-----|-----|
| +   | r | +  | r   | m   | r m |
| 38  |   | 12 | 19  | 18  |     |
| 59  |   | 6  | 28  | 23  |     |
| 71  |   | 9  | 4   | 1   |     |
| 7   | 1 | 2  | 5   | 7   |     |
| 18  | 2 | 3  | 38  | 9   |     |
| 44  |   | 19 | 38  | 18  | 4   |
| 47  |   | 11 | 32  | 31  |     |
| 17  |   | 13 | 10  | 0   |     |
| —   | — | —  | —   | —   | —   |
| 301 | 3 | 75 | 174 | 107 | 4   |

(b) Wild type (+), swollen (s), and miniature (m) males.

| ♀   |   |   | ♂   |     |     |     |
|-----|---|---|-----|-----|-----|-----|
| +   | s | m | +   | s   | m   | m s |
| 62  |   |   | 10  | 9   | 4   |     |
| 15  |   | 1 | 7   | 8   | 3   |     |
| 41  |   |   | 31  | 33  | 20  |     |
| 50  |   |   | 23  | 53  | 46  |     |
| 50  |   |   | 47  | 50  | 27  |     |
| 33  | 5 |   | 3   | 2   | 14  |     |
| 5   |   |   | 3   | 8   | 7   | 2   |
| 10  |   |   | 10  | 17  | 13  |     |
| 25  |   |   | 20  | 27  | 20  |     |
| 54  | 1 |   | 34  | 34  | 29  | 1   |
| 64  |   |   | 8   | 15  | 15  | 3   |
| 9   |   |   | 16  | 29  | 12  |     |
| —   | — | — | —   | —   | —   | —   |
| 418 | 6 | 1 | 212 | 285 | 210 | 6   |

(c) Round, swollen, and miniature males.

| ♀   |   |    |   | ♂  |     |     |     |     |     |
|-----|---|----|---|----|-----|-----|-----|-----|-----|
| +   | r | s  | m | +  | r   | s   | m   | r s | r m |
| 33  | 5 |    |   | 1  | 25  | 14  | 19  | 3   | 2   |
| 4   |   |    |   | 6  | 6   | 10  | 4   |     |     |
| 63  |   | 1  |   | 1  | 24  | 18  | 23  |     |     |
| 85  |   | 2  |   | 5  | 20  | 11  | 9   |     |     |
| 55  |   |    | 2 | 4  | 26  | 4   | 33  |     | 3   |
| 47  |   | 21 |   | 10 | 8   | 13  | 2   | 4   |     |
| 17  |   |    |   | 2  | 25  | 26  | 12  | 1   |     |
| 53  |   |    |   | 3  | 26  | 5   | 16  | 3   |     |
| 35  |   |    |   | 1  | 16  | 16  | 12  |     |     |
| 43  |   |    |   | 4  | 12  | 13  | 3   |     |     |
| 20  |   |    |   | 2  | 17  | 12  | 4   |     |     |
| 7   |   |    |   | 3  | 11  | 1   | 0   |     |     |
| —   | — | —  | — | —  | —   | —   | —   | —   | —   |
| 462 | 5 | 24 | 2 | 42 | 216 | 143 | 137 | 11  | 5   |

for the next division, but only the upper set in each case is shown here. It will be noted that the small autosomal V appears in both figures, 1a and 1b, but there are four rods in the oocyte figure and only three in the polar body. The extra rod seemed to pass at random into the polar body or into the second oocyte, as concluded after examining many eggs from a single female. In eggs from this digenic female only one limited chromosome was to be found. As is usual in *coprophila*, it was found to go either into the first polar body or into the second oocyte. In figure 1a it is with the five chromosomes in the second oocyte. In figure 2b it is seen with the five other chromosomes in the first polar body. Figures 2a and 2b were made from the second oocyte and the first polar body respectively, of another egg from the same female whose egg chromosomes are represented in figure 1. Here the two sex chromosomes (two of the rods) are passing out into the polar body, leaving one in the second oocyte, and hence one in the female pronucleus. Only the upper groups of the split chromosomes are represented.



FIGURE 2.—Chromosomes in second oocyte (a) and in first polar body (b) in one egg from the same digenic female. Only the upper group of split chromosomes is represented. Feulgen preparation.

### *Selective segregation*

The data obtained from experiments in which digenic females from successive generations were out-bred to mutant males from a monogenic stock seem to indicate that selective segregation obtains to an appreciable degree. Virgin digenic females were mated to males from a monogenic stock transmitting the mutant character round. Likewise virgin females from the first generation progenies were backcrossed to swollen males from a monogenic stock. Digenic females from these second generation progenies were out-bred to miniature males from monogenic stocks. The second generation progenies are recorded in table 3, third generation progenies in table 4 and fourth generation progenies are given in table 2.

From table 3 it is seen that only 19.46 percent of the second generation males showed the character round. Since round is sometimes carried concealed this percentage is undoubtedly a little low. But even after allowing for this inaccuracy the number of mutant males is still small for a random segregation of the three sex chromosomes carried in the germ line of the mothers of these flies. The character swollen, however, always shows in homozygous condition, and, as is evident in table 4, selective segregation obtains in maturation of eggs from digenic females carrying three distinc-



tive chromosomes, tagged with the wild type, round and swollen genes respectively. Here only 22.55 percent of the males in the third generation showed the swollen character. The class of miniature males in the fourth generation is not appreciably smaller than the other classes. However, outbreeding to miniature constituted the third successive generation in which

TABLE 3  
*Bisexual progenies from heterozygous round females mated to swollen males from a monogenic stock. Generation 2.*

| ♀     |   | ♂     |     |
|-------|---|-------|-----|
| +     | r | +     | r   |
| 8     |   | 33    | 9   |
| 82    |   | 0     | 0   |
| 74    |   | 23    | 4   |
| 55    |   | 21    | 6   |
| 23    |   | 25    | 3   |
| 13    |   | 49    | 3   |
| 37    |   | 86    | 12  |
| 20    |   | 16    | 9   |
| 61    |   | 50    | 5   |
| 44    | 1 | 80    | 21  |
| 30    |   | 62    | 23  |
| 11    |   | 7     | 1   |
| 27    |   | 24    | 2   |
| 32    |   | 17    | 7   |
| 49    |   | 51    | 14  |
| 13    |   | 5     | 4   |
| 17    |   | 25    | 7   |
| 32    |   | 27    | 5   |
| 36    |   | 25    | 9   |
| 14    |   | 6     | 2   |
| 53    | 2 | 65    | 33  |
| 54    |   | 11    | 9   |
| 59    |   | 56    | 11  |
| 58    |   | 17    | 6   |
| 11    |   | 2     | 2   |
| 23    |   | 62    | 5   |
| 13    |   | 33    | 0   |
| 16    |   | 17    | 0   |
| 65    | 1 | 31    | 7   |
| 22    |   | 16    | 12  |
| 49    |   | 21    | 4   |
| <hr/> |   | <hr/> |     |
| 1133  | 4 | 1080  | 261 |

digenic females from this line of experimentation were bred to monogenic stock males.

This type of behavior suggests that at maturation of the eggs synapsis between and separation of the two maternally derived chromosomes more often occurred, so that the paternally derived one, represented by the small class of males, more often accompanied one of the other two. In

19 to 22 percent of the cases, however, synapsis between and separation of the paternally and one of the maternally derived chromosomes took place, the other maternally derived X accompanying the latter, so that whatever force was at work here in bringing about this selection was not, by any means, absolute.

If selective segregation should account for a small class of males, the reciprocal class of females should also be small. So far as the evidence goes, the small classes of females support the hypothesis of selective segregation of the two maternally derived chromosomes.

TABLE 4

*Bisexual progenies from females, recorded in table 3, mated to miniature males from a monogenic stock. Generation 3.*

| TYPE 1 |     |   |    | TYPE 2 |   |     |     |     |    |
|--------|-----|---|----|--------|---|-----|-----|-----|----|
| ♀      | ♂   |   |    | ♀      | ♂ |     |     |     |    |
| +      | +   | r | s  | +      | r | +   | r   | s   | rs |
| 28     | 31  | 0 | 7  | 40     |   | 39  | 19  | 15  | 1  |
| 19     | 13  | 0 | 11 | 14     | 1 | 4   | 4   | 5   |    |
| 16     | 21  | 0 | 7  | 19     |   | 5   | 5   | 6   |    |
| 20     | 23  | 0 | 0  | 20     |   | 9   | 5   | 2   |    |
| 11     | 11  | 0 | 3  | 18     |   | 9   | 12  | 11  |    |
| 10     | 9   | 0 | 2  | 13     |   | 9   | 3   | 1   |    |
| 15     | 11  | 0 | 9  | 44     |   | 19  | 36  | 17  |    |
| 10     | 11  | 0 | 2  | 35     |   | 32  | 15  | 13  | 1  |
| 38     | 28  | 0 | 2  | 60     |   | 10  | 10  | 11  | 2  |
| 11     | 30  | 0 | 7  | 29     |   | 48  | 45  | 0   |    |
| —      | —   | — | —  | 44     |   | 10  | 19  | 15  | 1  |
| 178    | 188 | 0 | 50 | 50     |   | 0   | 40  | 14  | 1  |
|        |     |   |    | 21     |   | 0   | 19  | 11  |    |
|        |     |   |    | 20     |   | 8   | 8   | 8   | 1  |
|        |     |   |    | 17     |   | 17  | 16  | 4   |    |
|        |     |   |    | 17     |   | 9   | 10  | 4   |    |
|        |     |   |    | 13     |   | 8   | 6   | 4   |    |
|        |     |   |    | 20     |   | 10  | 5   | 6   |    |
|        |     |   |    | 19     |   | 5   | 5   | 6   |    |
|        |     |   |    | 11     |   | 5   | 6   | 5   |    |
|        |     |   |    | 18     |   | 14  | 17  | 7   |    |
|        |     |   |    | 28     |   | 19  | 11  | 6   |    |
|        |     |   |    | 40     |   | 27  | 24  | 13  |    |
|        |     |   |    | —      | — | —   | —   | —   | —  |
|        |     |   |    | 610    | 1 | 316 | 340 | 184 | 7  |

The question is, what difference was there between the chromosomes of paternal derivation and the other two, maternally derived, or what similarity existed between the two of maternal derivation to cause this greater attraction of the two maternal chromosomes for each other. It seems that the most obvious difference is that for at least two generations the two maternally derived chromosomes had been together and subjected to the same cytoplasmic influences, while that from the father had been in this same environment for only a single generation. This might well

be sufficient to bring about a greater similarity and hence greater attraction between the two maternally derived X's. Since selective segregation against paternally derived chromosomes obtains completely in the first maturation division of male germ cells in *Sciara* (Mertz 1927), it is not surprising that such selection as is described in the maturation of eggs in the trisomic germ line of digenic females should be found.

Why, in the fourth generation progenies (table 2), there seemed to be no particular selective segregation can be explained by the same sort of argument. After repeated out-breeding the environment in the cytoplasm of the germ line of the digenic female may not have been unique and sufficiently different from that found in the germ line of the monogenic males, so that no two chromosomes would come to be more similar and attractive to each other than any other two.

#### *Bisexual factor*

It has been mentioned that some females in the bisexual strain revert to male producers. This has been particularly true at certain times of the year, usually in midsummer and again in midwinter. It was observed that females in bisexual progenies which showed a mutant character were almost invariably monogenic. This fact suggested, after a trisomic condition in the germ line of digenic females had been demonstrated, that a gene carried in one of the original sex chromosomes of the bisexual strain was responsible for the retention of the trisomic condition.

To test this point digenic females were out-bred to swollen males from a monogenic stock. All flies of the first generation were wild type. Virgin females from these progenies were backcrossed to swollen males and gave in bisexual progenies wild type females and wild type and swollen males in the second generation. The males appeared in a ratio of two wild type to one swollen, with the swollen class deficient in number. More than two-thirds of these wild type females from the second generation, backcrossed again to swollen males from a monogenic stock, gave bisexual progenies consisting of wild type and swollen females and males in the approximate ratio of 2:1 and 1:2 respectively. Such a group of progenies is recorded in table 5.

Since the wild type females in such a progeny were digenic for the most part and the swollen females were monogenic, it was postulated that a bisexual factor in the wild type chromosome effected the retention of the trisomic condition of the germ line. To test this point further it was necessary to obtain by means of a crossover a bisexual line in which the bisexuality was linked with a definite mutant character. Such material was at hand in two all-swollen digenic lines which had been obtained in the course of the experiments and also in the bisexual progenies showing three classes of mutant males.

To ascertain in which of three sex chromosomes in the females represented in table 3c the bisexual factor might be carried, virgin females from these progenies were mated to wild type males from a monogenic stock and to the various mutant brothers. The mother of these progenies had mated to swollen males which came from male progenies in the various lines of the experiments. In the next generation 29 out of 51 progenies were bisexual. Here round, swollen and miniature females appeared as well as wild type females. These mutant and wild type females were again mated to wild type males from a monogenic stock and to the various mutant brothers. Only wild type and swollen females gave bisexual progenies in the next generation, and with one exception, all progenies from wild type females contained a class of swollen males. Many of these

TABLE 5

*Bisexual progenies from heterozygous swollen females from second generation progenies, backcrossed to swollen males from a monogenic stock. Generation 3.*

| ♀   |     | ♂   |     |
|-----|-----|-----|-----|
| +   | s   | +   | s   |
| 19  | 7   | 10  | 16  |
| 59  | 29  | 14  | 32  |
| 36  | 6   | 24  | 26  |
| 40  | 13  | 31  | 45  |
| 15  | 2   | 10  | 12  |
| 28  | 13  | 10  | 15  |
| 32  | 3   | 25  | 32  |
| 28  | 21  | 14  | 20  |
| 64  | 23  | 4   | 14  |
| 18  | 4   | 8   | 20  |
| —   | —   | —   | —   |
| 339 | 121 | 150 | 232 |

bisexual progenies did not contain round and many did not contain a miniature class of males. No bisexual progeny was obtained from round or miniature females in any lines of the experiment. Since the bisexual factor must have been carried in one of the mutant chromosomes, and since swollen was the only mutant character common to all bisexual progenies (with the one exception), it may be concluded that the bisexual factor was now linked with that for swollen. The one exception to this was a progeny containing 44 wild type females, 16 wild type males, 12 round males and 18 miniature males. Sisters of the female giving this progeny gave bisexual progenies with a class of swollen males; therefore, her mother carried the swollen chromosome and hence the bisexual factor, if this chromosome was the one which carried the factor. This bisexual progeny may be accounted for in one of two ways: either, another cross-over had occurred so that the bisexual factor was now carried in the wild type, round or miniature chromosome; or, without the presence of the

bisexual factor the tendency for the third sex chromosome to be eliminated from the germ line was not great enough to effect the elimination.

The second explanation brings up another point in connection with the effect of the bisexual factor. It will be recalled that many females in stock, particularly at certain times of the year, revert to male-producers. In the course of this investigation, females, known to carry the bisexual factor, were found to produce male progenies. We must postulate, then, another factor which affects the trisomic condition of the germ line, and which is opposed to the effect of the bisexual factor. This is a tendency for one of the three X's to be eliminated from the germ line. Whether this is a general tendency of the cytoplasm to reduce the triplo-X state or whether there are modifying factors in other chromosomes which oppose the bisexual factor in the X is not known. At any rate, such a variable tendency must be effective. If this tendency is high, the third X chromosome may be eliminated from the germ line of potentially digenic females in spite of the presence of the bisexual factor; or if it is low, the elimination may not occur when the bisexual factor is absent. The fact that many male progenies are produced in two seasons of the year indicates that the variation of the strength of this tendency may be correlated with the seasons, being highest in mid-summer and in mid-winter. The tendency must have been low in the following instance: a swollen female, from a progeny showing wild type and swollen females and wild type and swollen males in the ratios of 28:13 and 10:15 respectively, gave an all-swollen, bisexual progeny with 51 females and 51 males. All the females tested from this progeny gave typically male progenies. Here we may conclude that the tendency for elimination of the third X from the germ line of the mother of this all-swollen, bisexual progeny was not high enough to eliminate it even in the absence of the bisexual factor, but that such a tendency did effect the elimination in her daughters.

Swollen females from two other all-swollen, digenic lines, already mentioned, were mated to wild type males from a monogenic stock. They gave only swollen offspring (with occasional wild type daughters). The results in later generations seemed to indicate that the bisexual factor was now linked with swollen. The evidence at hand then seems to support strongly the hypothesis of the bisexual factor and the opposing tendency for the elimination of the third sex chromosome from the germ line of the digenic females.

It has long been known that males from the bisexual strain may be mated to monogenic females without showing any apparent tendency to transmit the bisexual factor. This behavior would be expected, however, since the factor is ineffective unless it appears in the female whose germ line already carries three X chromosomes. Such females are found normally in the bisexual strain and not in the monogenic lines.

*Unisexual progenies*

It has been stated previously that females in the digenic line may revert to male producers. Such females appear in too great numbers to be classed as ordinary exceptional females in male progenies. Male progenies from such females always show two classes of males, indicating that, some time after the fertilization of the XX egg which produced the mother of the male progeny, one of the three sex chromosomes must have been eliminated from the germ line. Almost without exception the chromosome eliminated was one of the maternally derived chromosomes, that is, the male-producing female always gave a class of males representing the sex chromosome from her father, the other class of males representing either one of the two maternally derived chromosomes.

Very rarely in the bisexual stock a female progeny appears. In the experimental lines of this investigation 19 definitely female progenies were obtained from females in bisexual progenies. This is 19 out of 984 progenies a percentage of 1.9, which is probably no greater than would be found in as many progenies in the bisexual stock. Previous tests of females from female progenies (notes from METZ's laboratory) showed that they were all unisexual producers. Most progenies obtained in these tests were male, but occasionally a female progeny was again obtained. The females in these second unisexual progenies were, however, male producers. From one of the female progenies obtained in these experiments (82 females:14 males) 11 females were bred and gave three female and eight male progenies. Six females from one of these latter female progenies (45 females:2 males) gave three typically bisexual and three male progenies. Daughters of these digenic females were bred and gave many bisexual progenies. The question arose as to why an occasional female in the digenic line would be a female producer. No adequate explanation has been found. The following possibility may account for such behavior: in the maturation of germ cells from the mother of a female progeny selective segregation may prevail so that always a single X chromosome is thrown off in the first polar body leaving two in the egg.

This possibility is somewhat borne out by the fact that a few male progenies were obtained that showed three classes of males. In these cases the reverse would have taken place leaving always the one X in the second oocyte. One male progeny consisted of 30 round, 8 miniature, 40 round-swollen, and 3 swollen flies. The mother must have been carrying round, miniature, and round-swollen. The three swollen males may have carried round concealed. Miniature came from the grandfather and the small size of this class was undoubtedly due to selective segregation, which usually prevails in digenic females out-bred to a monogenic stock. Other male progenies appeared to have come from females with germ lines carrying

three X's, but in them three classes of males could not be identified absolutely. Suggesting further that selective segregation may obtain, it is not at all uncommon to find bisexual progenies which show sex ratios approaching 1:0 or 0:1. An examination of sex ratios in progenies in table 2 shows that, when three classes of males are identifiable, the sex ratios may show an excessively large percentage of females or of males. It is not intended here to draw any conclusions concerning the reasons for obtaining female progenies, but rather to suggest the above possibility and to present the evidence at hand.

*Chromosome elimination from somatic cells*

The fact was mentioned earlier that in both females and males in the bisexual line the paternal sex chromosomes (two sister halves of the "precocious" chromosome) are eliminated from the somatic cells in early development. (This is the case also in the developing males in monogenic lines.) The fact that practically no swollen females were found in progenies from heterozygous swollen mothers, backcrossed to swollen, indicated this. Further evidence for this point was obtained from the progenies of digenic swollen females mated to wild type males. Table 6 shows a

TABLE 6  
*Progenies from four digenic swollen females mated  
to wild type males.*

| ♀ |    | ♂ |     |
|---|----|---|-----|
| + | s  | + | s   |
| 0 | 6  | 0 | 9   |
| 3 | 9  | 0 | 38  |
| 1 | 23 | 0 | 27  |
| 1 | 20 | 0 | 27  |
| — | —  | — | —   |
| 5 | 58 | 0 | 115 |

series of such progenies. Here almost all the females were swollen and the two swollen chromosomes in the somatic cells must have been of maternal origin. Since it is known that the digenic female has three sex chromosomes in her germ line, this seems to be the most valid interpretation. Without this knowledge the plausible explanation would be on the basis of non-disjunction. Some indication of non-disjunction was found in a few cases in the course of the investigation, but these were rare.

From table 6 it is seen that five out of 63 females were wild type. One of two things may have happened to account for these wild type females. Either they came from one-X eggs and are, therefore, comparable to exceptional females in a male progeny, or they came from two-X eggs and one of the maternally derived X chromosomes was eliminated instead of of the usual paternal one.

*Origin of the bisexual line*

It has been shown by BRIDGES (1913) and others that non-disjunction of the X chromosome may occur in the maturation of the egg in *Drosophila*. As a result of this the female pronucleus contains two sex chromosomes instead of the usual one and when such an egg is fertilized by an X-bearing sperm, three X's are to be found in the fusion nucleus, a condition referred to as triplo-X. When such a fertilized egg develops, a rare occurrence in *Drosophila*, a superfemale is produced. The soma, as well as the germ line, is trisomic.

In the case of *Sciara*, where elimination of the paternal X's (sister derivatives of the "precocious" chromosome) from the somatic cells regularly takes place in eggs from XX females, which normally develop into males, and where one of these sister derivatives is regularly eliminated from the germ line, non-disjunction would bring about a three-X condition of the germ line, but the soma would contain two sex chromosomes of maternal origin, and would, therefore, be female. Although elimination of one of the paternally derived X's from the germ line has not yet been observed, one of these must be thrown out sometime during development. In order that, after non-disjunction, the two maternally derived X's and one of the paternally derived ones should be kept in the germ line, it seems, from the results of the present investigation, that the bisexual factor must be present in one of the sex chromosomes; otherwise one of the three X chromosomes is eliminated from the germ line, reducing the number to the usual two. Likewise it seems that if the bisexual line arose as a result of non-disjunction, the bisexual factor was carried in at least one of the X's of the original female.

*Sex determination*

In developing eggs from XX (not X'X) females in the monogenic lines of *Sciara coprophila* the two sister derivatives of the "precocious" chromosome are regularly eliminated, leaving the soma with one X, and, therefore, a male develops. It has been shown by this investigation that the usual type of elimination in eggs from XX females, that is, elimination of both halves of the "precocious" chromosome, prevails in developing embryos from digenic females of the bisexual line, whether these embryos are developing as females or as males. Since this type of elimination in the monogenic line brings about the development of the male, and since in the digenic line after the same type of elimination females as well as males regularly develop, it must be concluded that the sex of the individual here is dependent upon not only the type of elimination, but some other points as well.

In the course of this investigation it has been shown that the digenic



females carry three sex chromosomes in their germ lines, two of maternal origin and one of paternal origin. At maturation two types of ovoids are formed, one with two X chromosomes and one with one X. Females, then, develop after fertilization of ovoids which have two X chromosomes. It may, therefore, be concluded that in the digenic line the type of maturation, that is, whether one X or two X's appear in the ovoid, influences the sex of the resulting individual.

METZ (1934) has suggested that the two sister derivatives of the "precocious" chromosome brought into the egg by the sperm may be functionally different, that one may have been altered during the second spermatocyte division, when it was mechanically distorted, so that in the development of the fertilized egg it may function as a "Y." He suggested that in the germ line of developing male embryos the altered X (now a functional "Y") is retained, the unaltered one being eliminated, and that development of the testes depends on the chromosome constitution left, that is, X"Y". In the female embryos one of these sister derivatives of the "precocious" chromosome is eliminated from the female somatic cells, and METZ suggested that the one eliminated from the germ line may be identical with that eliminated from the soma, although the elimination from the germ line is much later in ontogeny. In the case of the female, it is suggested that the unaltered X or derivative of the "precocious" chromosome is retained both in the soma and in the germ line. If this be the true interpretation, the germ line and soma differentiate independently of each other as has been found to be the case in most insects.

The question arises as to what determines which of these two sister derivatives of the "precocious" chromosome is to be eliminated from the germ line; is it the same factor which controls the elimination from the soma, namely the zygotic chromosome constitution of the mother, or since elimination from the germ line is subsequent to that from the soma, is the chromosome constitution of the soma after elimination the controlling factor? If the type of elimination from both soma and germ line in males of the monogenic lines depends upon the same factor, namely the XX (not X'X) chromosome complex of the mother, then in the digenic line where the same type of elimination from somatic cells takes place in both females and males, the same derivative of the "precocious" chromosomes would be expected to be retained in the germ line of both sexes. If it is the altered half which is retained in the male, the one kept in the germ line of the female should also be the altered one. Yet this paternally derived X in the germ line of these digenic females acts as an ordinary X and not as a "Y," as indicated by its later behavior.

If, therefore, the two halves of the "precocious" chromosome are functionally different, it seems probable that the constitution of the soma after

elimination is the controlling factor determining which half is to be retained in the germ line. Since this differential idea was proposed to account for independent differentiation of the germ cells, it seems unnecessary if the type of elimination of chromosomes depends on the somatic chromosome constitution.

#### ACKNOWLEDGMENT

I am deeply indebted to Professor C. W. Metz for suggesting this problem, for his guidance and helpful advice throughout the investigation, for his generosity in furnishing flies from his laboratory, and for making accessible data on the "Bisexual" line of *Sciara coprophila* obtained by him and his colleagues.

#### SUMMARY

1. The germ line of the digenic females carries three sex chromosomes instead of the usual two.
2. At maturation two kinds of eggs are formed, one with two X chromosomes and the other with one. After fertilization, eggs of the former type develop into females and those of the latter type into males.
3. During the development of these three-X females the same type of elimination occurs as is found in the developing males from the XX male-producing females in the monogenic line; that is, the two sex chromosomes brought into the egg by the sperm are eliminated from the somatic cells and one is eliminated from the germ line of both males and females in the "bisexual" line.
4. There is a factor, the bisexual factor, in at least one of the sex chromosomes of the digenic female which is responsible for the retention of the trisomic (XXX) condition of the germ line.
5. There is a tendency for the third chromosome to be eliminated from the germ line of digenic females, which tendency opposes the effect of the bisexual factor.
6. At maturation of the eggs of the digenic female there is a tendency for synapsis to occur between the two maternally derived chromosomes.

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# THE HOMOLGY OF THE EYE COLOR GENES IN *DROSOPHILA MELANOGASTER* AND *DROSOPHILA PSEUDOOBSCURA* AS DETERMINED BY TRANSPLANTATION. II.

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Received November 9, 1937

## INTRODUCTION

THERE are two methods by which chromosome and gene homologies can be studied: direct cytological examination, especially with the giant salivary gland chromosomes, and comparison of genetic chromosome maps. The first method is limited to those species which can be crossed and which produce viable offspring. Recent examples of the application of this method are the comparisons of salivary gland chromosomes of hybrids between *Drosophila pseudoobscura* and *D. miranda* (DOBZHANSKY and TAN 1936), and of *D. azteca* and *D. athabasca* (DOBZHANSKY and BAUER 1937). In species which cannot be crossed it is possible to apply only the genetical method. This involves establishing the homology of corresponding genes by the similarity of their phenotypical effects, and by comparison of their positions relative to other homologous neighboring genes. Attempts at such comparison were made early in *Drosophila* genetical work (MORGAN, BRIDGES and STURTEVANT 1925). More recently DONALD (1936) and STURTEVANT and TAN (1937) have used this method to make a detailed comparison of the linkage maps of *D. melanogaster* and *D. pseudoobscura*. The homology of genes in these species is based only upon the phenotypic effect. In a number of instances the comparisons are complicated because different genes produce similar effects.

We pointed out recently (1937) that the study of the reaction of eye discs in transplantation experiments can give useful information, especially for differentiating those genes which produce phenotypically similar effects. If in transplantation experiments the behavior of two genes is essentially the same, it may be concluded that they are homologous.

We have already established (1937) the homology of the genes "orange" *D. pseudoobscura* and "cinnabar" *D. melanogaster*, "vermillion" *D. pseudoobscura* and "vermillion" *D. melanogaster*, and "claret" *D. pseudoobscura* and "claret" *D. melanogaster*. It was pointed out that the two species differ quantitatively but not qualitatively in the reaction of implanted "wild type" anlagen. The behavior was always consistent with the supposition that *D. pseudoobscura* supplies less *v*<sup>+</sup> and *cn*<sup>+</sup> substances than does *D.*

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*melanogaster*. This is related to the fact that "wild type" *D. pseudoobscura* behaves in a manner identical to that of the recessive gene "clot" of *D. melanogaster*. We concluded that the gene "clot" or one similar to it is homozygous in *D. pseudoobscura* and is replaced in *D. melanogaster* by its "wild type" allele.

The present paper presents data obtained from intra- and inter-specific transplantation. It provides further evidence in support of the above conclusions and a general account of the behavior of all known eye color genes in *D. pseudoobscura*. On the basis of the new data we have established the homology of the known eye color genes in *D. pseudoobscura* and *D. melanogaster*.

#### METHODS AND MATERIAL

The methods used are described in our previous paper (1937). We have shown there that the difference in time of development of the two species is immaterial to the results obtained, as the host controls the period during which the implant undergoes pigmentation. The list of eye color mutants used in the present study is given here in alphabetical order with the standard mutant symbols:

| <i>D. pseudoobscura</i>                     | <i>D. melanogaster</i>  |
|---|---|
| <i>ca</i> —claret                           | <i>bw</i> —brown  |
| <i>cn</i> —cinnabar                         | <i>ca</i> —claret   |
| <i>mg</i> —magenta                          | <i>car</i> —carnation   |
| <i>or</i> —orange                           | <i>cd</i> —cardinal   |
| <i>p</i> <sup>2</sup> —pink-2 (pink-allele) | <i>cl</i> —clot   |
| <i>rb</i> —ruby                             | <i>cm</i> —carmine  |
| <i>se</i> —sepia                            | <i>cn</i> —cinnabar   |
| <i>st</i> —scarlet                          | <i>g</i> —garnet  |
| <i>v</i> —vermillion                        | <i>Hn</i> <sup>r</sup> —Henna <sup>r</sup> (recessive allele of <i>Hn</i> )         |
| <i>w</i> —white                             | <i>p</i> —pink  |
| <i>w</i> <sup>e</sup> —eosin (white allele) | <i>p</i> <sup>r</sup> —peach (pink allele)  |
| +—"wild type" <i>D. pseudoobscura</i>       | <i>rb</i> —ruby   |
| (Texas, race A)                             | <i>se</i> —sepia  |
|   | <i>st</i> —scarlet  |
|   | <i>v</i> —vermillion  |
|   | <i>w</i> —white   |
|   | <i>w</i> <sup>e</sup> —eosin (white allele)   |
|   | <i>w</i> <sup>a</sup> <i>cn</i> —apricot (white allele) cinnabar (double recessive) |
|   | <i>w</i> <sup>a</sup> <i>v</i> —apricot vermillion (double recessive)               |
|   | +—"wild type" <i>D. melanogaster</i>  |
|   | (Oregon-R-c)  |

Descriptions of these mutant genes are given by MORGAN, BRIDGES and STURTEVANT (1925) for *D. melanogaster* and by STURTEVANT and TAN (1937) for *D. pseudoobscura*. We shall refer to the mutant genes only by their symbols, but since the same symbol has often been used in both species indiscriminately, we shall distinguish the *D. melanogaster* and *D. pseudoobscura* symbols by the suffixes (m) and (p) respectively.

All of our *D. pseudoobscura* strains, with three exceptions, contained only a single mutant gene. The exceptions are: *pr*(p), carrying also beaded, yellow, short and Bare; *se*(p), carrying also beaded, miniature and snap; and *w<sup>e</sup>*(p) carrying also echinus and yellow. Since none of these affects the color of the eyes, their presence presumably did not affect the results. These stocks are therefore referred to by the symbols for the eye color only.

The stocks of *D. pseudoobscura* belong to race A, with the exception of "cinnabar," "orange" and "scarlet" which belong to race B.

#### THE BEHAVIOR OF THE EYE COLOR GENES AS DETERMINED BY TRANSPLANTATION

Table 1 presents diagrammatically the results obtained by the study of the eye transplants. The donor was always a female larva in its third instar (prepupa stage), the host a female or a male larva of the same age. Black discs in the table indicate a non-autonomous development of pigmentation. That means that the implant develops an eye color similar to that of the host, or in the cases of *v*(p), *v*(m), *or*(p), *cn*(m), *rb*(p), *g*(m) and *car*(m) develops the "wild type" eye color. Unshaded circles indicate autonomous development of pigmentation. Discs with a black sector ( $\frac{1}{2}$ - $\frac{3}{4}$ ) indicate, a partly non-autonomous development, where the implant is intermediate in color between the two controls; or in the seven mutants listed above, that the implant is intermediate between the "wild type" and the control implants in the donor larvae themselves.

Because of the complex interrelations between the different eye color genes considered in this paper, we have arranged the data in seven groups (a-g). The genes in each group have (a) identical relationship with each other; (b) the same type of relationship with the genes of any other group; (c) or apparently differ only quantitatively in either of the two properties. In table 1 these groups are separated by double lines.

#### *The behavior of the eye color genes of D. pseudoobscura in intraspecific transplantation experiments*

a) *The + group.* If + and *w<sup>e</sup>* eye discs are implanted into different hosts, the developing eye is affected by *ca*, *mg*, *pr*, and *p<sup>2</sup>* in such a way that the final pigmentation is very like the host eye color (*ca* and *mg*), or

| HOST<br>IMPL                | + <sub>P</sub>  | CL <sub>M</sub> | HN <sub>M</sub> <sup>f</sup> | + <sub>M</sub>  | W <sub>P</sub> <sup>*</sup> | ST <sub>P</sub> | ST <sub>M</sub> | CN <sub>P</sub> | CD <sub>M</sub> | RB <sub>P</sub> | G <sub>M</sub> | CAR <sub>M</sub> | RB <sub>M</sub> | CM <sub>M</sub> |
|-----------------------------|-----------------|-----------------|------------------------------|-----------------|-----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|------------------|-----------------|-----------------|
| + <sub>P</sub>              | ○ <sub>26</sub> | ○ <sub>10</sub> | ● <sub>3</sub>               | ● <sub>12</sub> | ○ <sub>17</sub>             | ○ <sub>8</sub>  |                 | ○ <sub>12</sub> |                 | ● <sub>6</sub>  | ● <sub>3</sub> | ● <sub>5</sub>   | ● <sub>7</sub>  | ○ <sub>3</sub>  |
| CL <sub>M</sub>             | ○ <sub>5</sub>  | ○ <sub>3</sub>  | ○ <sub>6</sub>               | ○ <sub>3</sub>  |                             |                 |                 | ○ <sub>3</sub>  |                 | ● <sub>5</sub>  |                |                  | ○ <sub>3</sub>  |                 |
| HN <sub>M</sub>             | ○ <sub>4</sub>  |                 | ○ <sub>X</sub>               | ○ <sub>X</sub>  |                             |                 |                 |                 |                 |                 |                |                  |                 |                 |
| + <sub>M</sub>              | ○ <sub>6</sub>  | ○ <sub>3</sub>  | ○ <sub>X</sub>               | ○ <sub>16</sub> | ○ <sub>5</sub>              |                 | ○ <sub>X</sub>  | ○ <sub>4</sub>  | ○ <sub>X</sub>  | ● <sub>4</sub>  | ○ <sub>X</sub> | ○ <sub>X</sub>   | ○ <sub>X</sub>  | ○ <sub>X</sub>  |
| W <sub>P</sub> <sup>*</sup> | ○ <sub>5</sub>  | ○ <sub>4</sub>  |                              | ○ <sub>2</sub>  | ○ <sub>13</sub>             |                 |                 |                 |                 |                 |                |                  |                 |                 |
| ST <sub>P</sub>             | ○ <sub>4</sub>  |                 |                              |                 |                             | ○ <sub>3</sub>  |                 | ○ <sub>1</sub>  |                 | ○ <sub>5</sub>  |                |                  |                 |                 |
| ST <sub>M</sub>             |                 |                 | ○ <sub>X</sub>               |                 |                             |                 | ○ <sub>1</sub>  |                 | ○ <sub>5</sub>  |                 |                |                  |                 |                 |
| CN <sub>P</sub>             | ○ <sub>15</sub> | ○ <sub>10</sub> | ○ <sub>1</sub>               |                 |                             | ○ <sub>1</sub>  |                 | ○ <sub>6</sub>  |                 | ○ <sub>4</sub>  |                |                  |                 |                 |
| CD <sub>M</sub>             |                 |                 | ○ <sub>X</sub>               |                 |                             |                 | ○ <sub>X</sub>  |                 | ○ <sub>4</sub>  |                 |                |                  |                 |                 |
| RB <sub>P</sub>             | ○ <sub>3</sub>  | ○ <sub>4</sub>  | ○ <sub>3</sub>               | ○ <sub>3</sub>  |                             | ● <sub>2</sub>  |                 | ● <sub>3</sub>  |                 | ○ <sub>11</sub> |                |                  |                 |                 |
| G <sub>M</sub>              | ○ <sub>2</sub>  |                 | ○ <sub>X</sub>               |                 |                             |                 |                 |                 |                 | ○ <sub>2</sub>  |                |                  |                 |                 |
| CAR <sub>M</sub>            | ○ <sub>8</sub>  |                 | ○ <sub>X</sub>               |                 |                             |                 |                 |                 |                 |                 | ○ <sub>X</sub> |                  |                 |                 |
| RB <sub>M</sub>             | ○ <sub>4</sub>  |                 | ○ <sub>X</sub>               |                 |                             |                 |                 |                 |                 |                 |                |                  | ○ <sub>3</sub>  |                 |
| CM <sub>M</sub>             | ○ <sub>6</sub>  |                 | ○ <sub>X</sub>               |                 |                             |                 |                 |                 |                 |                 |                |                  |                 | ○ <sub>X</sub>  |
| OR <sub>P</sub>             | ● <sub>6</sub>  | ● <sub>5</sub>  | ● <sub>3</sub>               | ● <sub>3</sub>  |                             | ● <sub>5</sub>  | ● <sub>1</sub>  | ● <sub>2</sub>  | ● <sub>2</sub>  | ● <sub>5</sub>  | ● <sub>5</sub> | ● <sub>4</sub>   | ● <sub>4</sub>  | ● <sub>1</sub>  |
| CN <sub>M</sub>             | ● <sub>2</sub>  | ● <sub>6</sub>  | ○ <sub>X</sub>               | ○ <sub>X</sub>  |                             |                 | ○ <sub>X</sub>  |                 | ○ <sub>X</sub>  | ○ <sub>X</sub>  | ○ <sub>X</sub> | ○ <sub>X</sub>   | ○ <sub>X</sub>  | ○ <sub>X</sub>  |
| V <sub>P</sub>              | ● <sub>15</sub> | ● <sub>7</sub>  | ● <sub>3</sub>               | ● <sub>3</sub>  | ● <sub>2</sub>              | ● <sub>6</sub>  |                 | ● <sub>4</sub>  |                 | ● <sub>3</sub>  | ● <sub>6</sub> | ● <sub>4</sub>   | ● <sub>5</sub>  |                 |
| V <sub>M</sub>              | ○ <sub>14</sub> | ○ <sub>4</sub>  | ○ <sub>X</sub>               | ○ <sub>X</sub>  |                             |                 | ○ <sub>X</sub>  |                 | ○ <sub>X</sub>  | ○ <sub>2</sub>  | ○ <sub>X</sub> | ○ <sub>X</sub>   | ○ <sub>2</sub>  | ○ <sub>X</sub>  |
| CA <sub>P</sub>             | ○ <sub>15</sub> | ○ <sub>3</sub>  | ○ <sub>10</sub>              | ○ <sub>3</sub>  |                             | ○ <sub>1</sub>  |                 | ○ <sub>2</sub>  |                 | ○ <sub>1</sub>  |                |                  |                 |                 |
| CA <sub>M</sub>             | ○ <sub>5</sub>  | ○ <sub>3</sub>  | ○ <sub>4</sub>               |                 |                             |                 |                 |                 |                 |                 |                |                  |                 |                 |
| MG <sub>P</sub>             | ○ <sub>5</sub>  | ○ <sub>3</sub>  | ○ <sub>5</sub>               | ○ <sub>25</sub> |                             | ○ <sub>5</sub>  |                 | ○ <sub>5</sub>  |                 | ○ <sub>11</sub> | ○ <sub>2</sub> | ○ <sub>6</sub>   |                 | ○ <sub>5</sub>  |
| PR <sub>P</sub>             | ○ <sub>6</sub>  | ○ <sub>5</sub>  | ○ <sub>7</sub>               | ○ <sub>5</sub>  |                             |                 |                 |                 |                 | ○ <sub>5</sub>  |                |                  |                 |                 |
| P <sub>P</sub> <sup>2</sup> | ○ <sub>5</sub>  | ○ <sub>7</sub>  | ○ <sub>4</sub>               | ○ <sub>23</sub> |                             |                 |                 |                 |                 | ○ <sub>4</sub>  |                |                  |                 |                 |
| BW <sub>M</sub>             |                 |                 | ○ <sub>X</sub>               | ○ <sub>4</sub>  |                             |                 |                 |                 |                 |                 |                |                  |                 |                 |
| P <sub>M</sub> <sup>p</sup> |                 |                 | ○ <sub>X</sub>               |                 |                             |                 |                 |                 |                 |                 |                |                  |                 |                 |
| SE <sub>P</sub>             | ● <sub>17</sub> | ● <sub>17</sub> | ● <sub>5</sub>               | ● <sub>11</sub> | ● <sub>4</sub>              | ● <sub>3</sub>  |                 | ● <sub>3</sub>  |                 |                 |                |                  |                 |                 |
| SE <sub>M</sub>             | ○ <sub>4</sub>  | ○ <sub>1</sub>  | ○ <sub>X</sub>               |                 |                             |                 |                 |                 |                 |                 |                |                  |                 |                 |

TABLE I

The sign X means experiments done by BEADLE and EPHRUSSI. For description see text.

| OR <sub>P</sub> CN <sub>M</sub> | V <sub>P</sub> V <sub>M</sub> | CA <sub>P</sub> CA <sub>M</sub> MG <sub>P</sub> | PR <sub>P</sub> P <sub>P</sub> <sup>2</sup> BW <sub>M</sub> P <sub>M</sub> <sup>p</sup> | SE <sub>P</sub> SE <sub>M</sub> |
|---------------------------------|-------------------------------|---|---|---------------------------------|
|                                 |                               |   |   |                                 |
|                                 |                               |   |   |                                 |
|                                 |                               |   |   |                                 |
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|                                 |                               |   |   |                                 |
|                                 |                               |   |   |                                 |
|                                 |                               |   |   |                                 |

TABLE I (continued)



intermediate between the host and donor eye colors (*pr* and *p*<sup>2</sup>). The light *w*<sup>e</sup> implant does not change in *pr* and *p*<sup>2</sup> as much as + does. In *or* and *v* hosts the + implant is changed to a slightly but distinctly brighter eye color than + controls. The change is more pronounced in *v* than in *or*. The *w*<sup>e</sup> implant remains unchanged in *or*, but is visibly changed in *v*. In *se* hosts, the + and *w*<sup>e</sup> implants develop a pinkish eye color, and become similar in appearance. + implanted in *rb* becomes intermediate between + and *rb*, and in *st* and *cn* it develops the original + eye color. It must be stated, however, that some of the eyes implanted into *cn* were brighter than the "normal" + implants. It is not clear from these experiments what effects age differences (younger and older + anlagen in *cn* hosts) might have.

The implantation of the eye discs of different mutants into + and *w*<sup>e</sup> results in an autonomous development of the eye color in all cases except those involving *or*, *v* and *se*. The data given in table 1 for these three genes show that when implanted into + and *w*<sup>e</sup> hosts, *v* and *or* optic discs give rise to a not quite completely "wild type" eye. Eye discs carrying the gene *se* develop in + and *w*<sup>e</sup> hosts, a new dark brownish-red eye color. The new eye color resembles neither the + nor the *w*<sup>e</sup> nor the *se* type, and apparently the amount of pigment in the new eye color type is not decreased.

+ in *w*<sup>e</sup>, and the reciprocal combination, develop autonomously. Discussion of the interrelations of host and donor will be postponed until we have referred to the other *D. pseudoobscura* groups, and to the homologous groups in *D. melanogaster*.

b) *The st group*. The *st* and *cn* implants behave autonomously in all experiments carried out by us.

If the host is *st* or *cn*, then *rb*, *or*, *v* and *se* implants develop non-autonomously. The behavior of + in *cn* has been mentioned above. If *rb*, *or* and *v* discs are implanted into *st* and *cn* they are changed almost completely to "wild type" eyes. With the exception of *or* in *st*, the different combinations are indistinguishable, and similar to the implants of *or* and *v* in the + group. The eye discs carrying the gene *or* implanted into *st* become distinctly lighter than *or* in +. A change in the direction of the new eye color obtained from *se* implanted into + (see + group) occurs also in the experiment *se* implanted into *st* or *cn* hosts.

The development is autonomous in the experiments *st* implanted into *cn* and in the reciprocal combination.

c) *The rb group*. As an implant the *rb* eye shows an autonomous development in the + group and in *pr*. In the *st* group it changes almost completely to "wild type." The *rb* implant in *or* becomes very like "wild type." The *rb* optic discs give rise in *v*, *ca* and *mg* to an eye color which is intermediate between ruby and + eye colors. In *se* the implanted *rb* anlage is definitely pale, resembling neither *rb* nor +.

Implantation of + into a *rb* host resulted in a non-autonomous development, and an eye color very similar to that of *rb* implanted into *v*. In the experiment *or* implanted into *rb* the implant eye color is like that of + in *rb*, but more reddish, and *v* implanted into *rb* is very close to "wild type." In *rb* hosts the *p*<sup>2</sup> implant shows a "brownish-red" eye color similar to but not as dark as *se* implanted into + (see + group). The eye color of *pr* implanted into *rb* is similar to, but lighter than, *se* implanted in *st* or *cn*.

d) *The v group*. We discuss *or* and *v* eye color genes together because in all cases in which the *or* eye discs give rise in the different *D. pseudoobscura* hosts to eye colors similar to "wild type," the *v* optic discs also develop non-autonomously to a + type. Nevertheless there is a distinct difference between the two genes, which cannot be explained by quantitative differences between them. Namely, *v* implanted into *or* gives rise to a "wild type" eye color; *or* implanted into *v* develops autonomously.

In +, *w*<sup>e</sup>, *st* and *cn* hosts optic discs carrying the genes *or* and *v* develop to eyes which are not completely "wild type." The implant *v* in *rb* becomes more nearly "wild type" than does *or*; the same is true for *v* and *or* implanted into *p*<sup>2</sup>, but here the change is not as great as in the preceding case. In *pr* the implanted *v* eye is indistinguishable from the implant of *v* in *p*<sup>2</sup>. However, the *or* anlagen develop autonomously in *pr*, if the implanted eye is of normal size. If the implanted optic discs give rise to small eye pieces a change to + is clearly noticeable. That means that *pr* supplies sufficient *cn*<sup>+</sup> substance for the change to take place if the implanted eye is small. In *ca* and *mg* both *or* and *v* discs develop autonomously. In *se* only the *v* anlage shows a slight change to +.

If *or* and *v* are used as hosts, *st*, *cn*, *ca*, *mg*, *pr* and *p*<sup>2</sup> optic discs develop autonomously. + shows a slight change to a brighter eye color in *or*, and a more marked change in the same direction in *v*. The anlage carrying *w*<sup>e</sup> changes only in *v*, and here to a lighter eye color. In *or* hosts the implant *rb* gives rise to a nearly "wild type" eye color; in *v* this change is not as strong and the eye is brighter. The implant *se* shows in *or* hosts an eye color which resembles very much *se* in +, but the implanted eye appears brighter. The color of the implant *se* in *v* is best described as intermediate between *se* and *v*. It has a slight orange tinge.

If *v* is implanted into *or* the anlage develops an eye identical to *v* in + host; *or* is autonomous in *v*.

e) *The ca group*. Our experiments have shown that *ca* and *mg* always develop autonomously when used as implants.

If *ca* and *mg* are used as hosts, only the implanted optic discs of the +, *rb* and *se* groups are non-autonomous in their development. The results of the experiments concerning +, *w*<sup>e</sup> and *rb* are mentioned above. If *se* is implanted into *ca*, then the eyes show a reddish color closely resembling

*ca* itself; implanted into *mg*, *se* eyes show a more pinkish color than the *se* controls.

In reciprocal *ca-mg* implantations the optic discs develop autonomously.

f) *The pr group*. The behavior of *pr* and *p*<sup>2</sup> resembles very much that of *ca* and *mg*. However, the development of *pr* and *p*<sup>2</sup> implanted in *rb* hosts is not entirely autonomous (see above: the *rb* group).

If *pr* and *p*<sup>2</sup> are the hosts, + and *w*<sup>e</sup> implants do not change as much as in the *ca* group. Eye discs carrying *se* implanted into *pr* or *p*<sup>2</sup> develop an eye color similar to that of *se* in *mg*. The behavior of optic discs carrying *or* or *v* when implanted in *pr* or *p*<sup>2</sup> is different from their behavior in *ca* or *mg* hosts. In the former cases they develop non-autonomously. The data are given above under d, the *v* group.

In the reciprocal experiments *pr* implanted into *p*<sup>2</sup> and *p*<sup>2</sup> implanted into *pr* the anlagen behave autonomously.

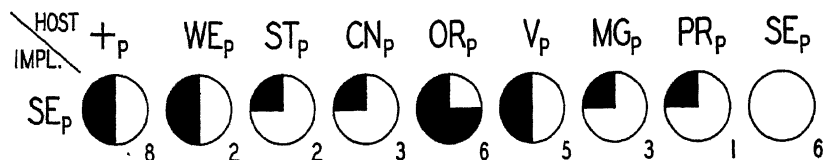


TABLE 2

*Pupal dissections of se(p) optic discs implanted into different hosts (see text).*

g) *The se group*. The behavior of the gene *se* is different in every respect from that of the other genes. If *se* is implanted into the + group the anlage gives rise to a new dark "brownish red" eye color. About the same eye color develops if *se* discs are implanted into the *st* group, but in this case the color is somewhat more *se*-like. In *or*, *se* changes to a color which is brighter but essentially of the same type as in +; in *v* it changes to an intermediate color (see above, d, the *v* group). In *ca* the eye color of the *se* implant proved to be more like that of the host than in *mg*, *pr* and *p*<sup>2</sup>. Because the *se* eye darkens very rapidly with age, we have always examined freshly hatched flies, and in a number of cases dissected out pupae shortly before they hatched. The data obtained from experiments of the latter kind are listed in table 2. The results here agree with those presented in table 1.

The development by the + and *w*<sup>e</sup> implants of a pinkish eye color is obvious in *se* hosts; *v* implanted into *se* hosts shows a slight change toward the + type. The other genes, with exception of *rb*, which has a pale eye color when implanted, behave autonomously in *se* hosts.

The observed behavior of all the genes within each of the seven groups listed above is *similar* in all experiments. In this sense, we may regard them as natural groups; only the *v* group is perhaps a composite. A com-

parison of the groups with each other is presented in table 3. The conclusions that may be drawn from the comparison are as follows.

a) The eye anlagen of the + group develop a color similar to that of the hosts when implanted into the *ca*, *pr* and *rb* groups; a new eye color in the *se* group, and a color distinctly brighter than normal in the *v* group. The development of the + group is autonomous only in the + group itself, and in the *st* group.

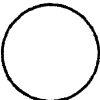
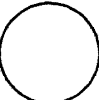





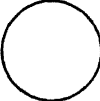
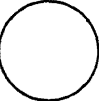
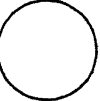
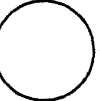
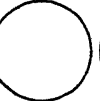

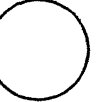
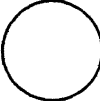

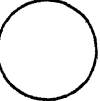







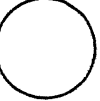
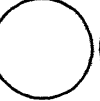


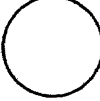
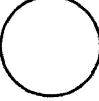
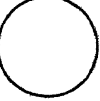
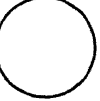




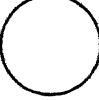











| HOST<br>IMPL. | +P  | ST  | RB  | V   | CA  | PR  | SE  |
|---------------|---|---|---|---|---|---|---|
| +P            |    |    |    |    |    |    |    |
| ST            |    |    |    |    |    |    |    |
| RB            |    |    |    |    |    |    |    |
| V             |    |    |    |    |    |    |    |
| CA            |   |   |   |   |   |   |   |
| PR            |  |  |  |  |  |  |  |
| SE            |  |  |   |  |  |  |  |

TABLE 3

Review of the behavior of the eye color gene groups within *D. pseudoobscura* (see text).

b) The *st* group always behaves autonomously.

c) The optic discs of the *rb* group implanted into the *st*, *v* and *ca* groups give rise to an eye color similar to the "wild type." The anlagen of the *rb* group develop a pale eye color when implanted into the *se* group. The eye discs carrying genes of the *rb* group develop autonomously only in hosts belonging to the *rb*, + and *pr* groups.

d) The *v* group shows a wild type eye color when implanted into the +, *st*, *rb*, *pr* and *se* groups; the development is autonomous only in the *ca* group.

e) The *ca* group always behaves autonomously.

f) The eye discs carrying genes of the *pr* group develop autonomously in all combinations studied, except when implanted into hosts belonging to the *rb* group. In the latter case, the implants develop an eye color resembling that of + implanted into *se*.

g) The *se* group changes the original color toward a dark "brownish-red" one in the + group, and to nearly the same color in the *st* group. When *se* is implanted into *v* the color of the implant becomes intermediate between *se* and *v*. The eye discs carrying genes of the *se* group, when implanted into *or* hosts, develop an eye color which is somewhat similar to that developed by the same implants into + hosts. In the *ca* and *pr* groups the color of the *se* implants is somewhat similar to that of the host eyes.

#### THE HOMOLOGOUS EYE COLOR GENES IN *D. MELANOGASTER* AND *D. PSEUDOOBSCURA*

a) *The + group* [+ (*p*), *cl*(*m*), *Hn*<sup>r</sup>(*m*), + (*m*), *w*<sup>e</sup>(*p*), *w*<sup>e</sup>(*m*)]. In our first paper (GOTTSCHIEWSKI and TAN (1937), we pointed out that the eye discs of "wild type" *D. pseudoobscura* behave in all transplantation experiments like those of the mutant clot (*cl*, Chrom. 2-16.5) of *D. melanogaster*. We concluded that the genetic difference between these two species involves, among others, a mutation at the clot locus. Additional data to support this conclusion are presented in table 1. Among the mutant genes known in *D. melanogaster* there is one, Henna (*Hn* Chrom. 3-23.0), which has a superficial resemblance to both *cl*(*m*) and + (*p*). Data are available however, to prove that + (*p*) is not homologous to *Hn*<sup>r</sup>(*m*). Indeed, the eye discs of + (*p*) implanted into *Hn*<sup>r</sup>(*m*) develop a brighter eye color than that developed by + (*p*) in + (*p*) and similar to + (*p*) implanted into + (*m*). Moreover, *v*(*p*) implanted into *Hn*<sup>r</sup>(*m*) develops completely non-autonomously, identical with *v*(*p*) implanted into + (*m*), while the development of *v*(*p*) in + (*p*) is only partly so.

Eosin and white mutants in *D. pseudoobscura* resemble greatly the similarly named mutants in *D. melanogaster*. In both species the white locus is sex-linked and is located in the proximity of the gene Notch, which in its distinct properties is the same in both species (STURTEVANT and TAN 1937). The behavior of *w*<sup>e</sup>(*m*) in the transplantation experiments of BEADLE and EPHRUSSI (1936) is similar to that of + (*m*). Our experiments within *D. pseudoobscura* show also essentially the same behavior of *w*<sup>e</sup>(*p*) and + (*p*) in respect to autonomous or non-autonomous development. The quantitative differences in our implants of + (*p*) and *w*<sup>e</sup>(*p*) into

$v(p)$ ,  $pr(p)$  or  $p^2(p)$  are not significant. In the first place if a dilution of eye pigment takes place a slight change in the  $w^e$  implants is more difficult to notice than in the "wild type" implants, due to the originally lighter eye color. Consequently, the change to a lighter eye in  $v(p)$  hosts may be of the same degree in  $w^e(p)$  as in  $+(p)$  anlagen. In the second place the eye colors of  $w^e(p)$ ,  $p^2(p)$  and  $pr(p)$  implants are much more alike than those of  $+(p)$ ,  $p^2(p)$  and  $pr(p)$ . We may conclude that  $w^e(p)$  is homologous to  $w^e(m)$ .

b) *The st group* [ $st(p)$ ,  $st(m)$ ,  $cn(p)$ ,  $cd(m)$ ]. A group of eye color mutants comprising  $st(p)$ ,  $cn(p)$ ,  $or(p)$  and  $v(p)$  shows a great resemblance to the  $st(m)$ ,  $cd(m)$ ,  $cn(m)$  and  $v(m)$  group of *D. melanogaster* mutants. Moreover, the members of each of these groups are so similar to each other that they can hardly be distinguished by simple inspection. Only  $cn(p)$  and  $cd(m)$  show a slight darkening with age, which sets them somewhat apart from the rest. The transplantation methods furnish more critical data for discrimination between these eight mutants. Indeed, the eye discs of  $st(p)$ ,  $st(m)$ ,  $cn(p)$  and  $cd(m)$  implanted into hosts of the  $+$  group develop autonomously, whereas  $or(p)$ ,  $cn(m)$ ,  $v(p)$  and  $v(m)$  change in the direction of the "wild type" eye color.

An attempt was made to differentiate between  $st(p)$ ,  $cn(p)$ ,  $st(m)$  and  $cd(m)$  by studying their influence on the production of the  $v^+$  and  $cn^+$  substances. The results are so far not self-consistent. According to BEADLE and EPHRUSSI (1936),  $v(m)$  and  $cn(m)$  develop non-autonomously when implanted into  $st(m)$  or  $cd(m)$  hosts. Later, BEADLE and EPHRUSSI (1937) found that  $cd(m)$  implanted into  $w^{av}(m)$  fails to change the eye color of the host, but such a change does take place if  $st(m)$  is implanted into  $w^{av}(m)$  hosts. The implantation of  $cd(m)$  into  $w^{acn}(m)$  hosts results in the host's eye developing a color intermediate between  $w^{acn}$  and  $w^a$ , but closer to  $w^{acn}$ . Similarly, the implantation of  $st(m)$  into  $w^{acn}(m)$  hosts changes the eye color of the host to one intermediate between  $w^{acn}$  and  $w^a$ . This intermediate color is apparently closer to  $w^a$  than that obtained in the experiments with  $cd(m)$ . These results indicate that  $st(m)$  supplies more  $v^+$  and  $cn^+$  substances than does  $cd(m)$ . Our experiments within *D. pseudoobscura* showed, at least for the supply of  $cn^+$  substance, the reverse result. Apparently more substance is supplied by  $cn(p)$  to  $or(p)$  than by  $st(p)$ . The developing implant eye color of  $or(p)$  is distinctly lighter in a  $st(p)$  than in a  $cn(p)$  host. To check these results we injected body fluid of about one day old pupae of  $cn(p)$  and  $st(p)$  into  $w^{av}(m)$  larvae. We found that  $w^{av}(m)$  host flies showed darker  $w^a$ -like eyes, if we injected the body fluid of  $cn(p)$ , than in the analogous experiment with  $st(p)$  body fluid. In the latter case the eyes of the host were like those of  $w^{av}(m)$  flies into which the body fluid from  $w^{av}(m)$  stock itself was injected.

We may conclude that *st*(p), *st*(m), *cn*(p) and *cd*(m) behave alike. Our experiments are not extensive enough to establish a distinct difference between these genes. The presence of the gene *clot*(m) in *D. pseudoobscura* is of great importance for the amount of *v*<sup>+</sup> and *cn*<sup>+</sup> substances. Hence, the darkening effect in *cn*(p) and *cd*(m) indicates the homology of *cn*(p) and *cd*(m). The essentially similar behavior of the remaining comparable genes of this group, namely *st*(p) and *st*(m), indicates that *st*(p) is homologous to *st*(m).

c) *The rb group* [*rb*(p), *g*(m), *car*(m), *rb*(m), *cm*(m)]. The pinkish eye colors produced by the *rb*, *ca*, *mg*, and *p* mutants in *D. pseudoobscura* resemble the *rb*, *g*, *car*, *cm*, *ca* and *p* eye colors of *D. melanogaster*. With the aid of transplantation experiments, these mutants can be divided into three distinct classes: the *rb*, *ca* and *pr* groups. The basic difference between these groups is that the implants containing the genes of the *rb* group develop non-autonomously and assume the "wild type" coloration if implanted into hosts carrying genes of the *st* and *v* groups; the implants carrying genes of *ca* and *pr* groups under similar conditions develop autonomously.

Although the possibility is not excluded that *cm*(m) may belong to the *rb* group, the homology between it and *rb*(p) is very doubtful. Indeed, *+*(p) develops autonomously when implanted into a *cm*(m) host, and in addition the behavior of *cm*(m) and of *rb*(p) is different, both when used as implants and as hosts, in experiments involving *or*(p) and *v*(p).

The behavior of *rb*(m) when implanted into hosts carrying *or*(p) differs from that of the other genes of its group. STURTEVANT and TAN (1937) concluded that "if it be granted that each arm of *pseudoobscura* is equivalent to one of *melanogaster*" (and we have no evidence that this conclusion is wrong) and "if the *pseudoobscura* sequence of the comparable loci" in each arm "is arbitrarily taken as an alphabetical one," then seven inversions are sufficient to turn the sequence in the XL chromosome of *D. pseudoobscura* into that of the X of *D. melanogaster*. They did not include in their calculations the gene *rb*(p). Assuming *rb*(p) to be homologous to *g*(m) or to *car*(m), still only seven inversions are required to get the identical sequence in both species. If we homologize *rb*(p) with *rb*(m) eight steps would be necessary, and more for homology with *cm*(m). Moreover, in the transplantation experiments the behavior of *g*(m) and *car*(m) agrees best with that of *rb*(p). However, a difference between *g*(m) and *car*(m) was obtained in our experiments. Implanted into *mg*(p) hosts, *g*(m) gives rise to an eye color in the implant which is intermediate between a pinkish and a "wild type" color, and indistinguishable from the *rb*(p) eye when implanted into *mg*(p). In the analogous experiment the *car*(m) optic discs develop more nearly autonomously. One additional piece of evidence favors

the homologizing of  $rb(p)$  with  $g(m)$  rather than with  $car(m)$ . The gene  $car(m)$  mutates very rarely, having been recorded only once as a mutant from X-ray treatments, whereas  $g(m)$  mutates relatively frequently. The mutations at the  $rb(p)$  locus are not infrequent either. Consequently, we are inclined to believe that  $rb(p)$  is homologous to  $g(m)$ .

d) *The v group* [ $or(p)$ ,  $cn(m)$ ,  $v(p)$ ,  $v(m)$ ]. The transplantation experiments mentioned above (the *st* group) serve to distinguish the genes  $st(p)$ ,  $st(m)$ ,  $cn(p)$  and  $cd(m)$  from  $or(p)$ ,  $cn(m)$ ,  $v(p)$  and  $v(m)$ . These genes have a close resemblance to each other. In our recent paper (GOTTSCHESKI and TAN, 1937) we concluded that  $or(p)$  is homologous to  $cn(m)$ , and  $v(p)$  to  $v(m)$ . The data presented in table 1 support these conclusions. We may add that in general the *D. pseudoobscura* genes seem to supply less  $cn^+$  and  $v^+$  substances than the *D. melanogaster* genes do. The only exception in *D. melanogaster* is the gene  $cl(m)$ , which behaves like *D. pseudoobscura* "wild type." In *D. pseudoobscura*, the exception seems to be  $rb(p)$ , which supplies at least as much of  $cn^+$  and  $v^+$  substances as the homologous gene in *D. melanogaster*,  $g(m)$ . We may conclude from the data listed under the *rb* and *pr* groups that  $or(p)$  requires less  $cn^+$ , and  $v(p)$  less  $v^+$  substance than  $cn(m)$  and  $v(m)$  respectively. Discussion of these relations is deferred until additional evidence with body fluid is obtained. We homologize  $or(p)$  with  $cn(m)$  and  $v(p)$  with  $v(m)$ .

e) *The ca group* [ $ca(p)$ ,  $ca(m)$ ,  $mg(p)$ ]. The characteristics distinguishing the *rb* and *ca* groups have been discussed above. We have presented (1937) the evidence proving that  $ca(p)$  is homologous to  $ca(m)$ . For the gene  $mg(p)$  which behaves essentially similarly to  $ca(p)$ , it has been so far impossible to find a homologue in *D. melanogaster*;  $mg(p)$  is located in XR(p),  $ca(p)$  in II(p) and  $ca(m)$  in IIIR(m), IIIR(m) being homologous to the second chromosome of *D. pseudoobscura*. Since  $+$ (m) is non-autonomous in  $ca(p)$ , and behaves similarly in  $ca(m)$ , but is intermediate in  $mg(p)$ , we conclude that  $ca(p)$  is homologous to  $ca(m)$ .

f) *The pr group* [ $pr(p)$ ,  $bw(m)$ ,  $p^2(p)$ ,  $p^2(m)$ ]. The genes  $pr(p)$  and  $p^2(p)$  behave alike with two exceptions. First, it seems that  $p^2(p)$  supplies proportionately more  $cn^+$  and less  $v^+$  substances than  $pr(p)$ , because  $or(p)$  implanted in  $pr(p)$  shows a change to "wild type" only in small eye pieces. In  $p^2(p)$  the effect is always recognizable;  $v(m)$  becomes more like the "wild type" if implanted into  $pr(p)$  than if implanted into  $p^2(p)$ . The other exception is that  $pr(p)$  in  $rb(p)$  changes only very slightly, but in analogous experiments with  $p^2(p)$  the effect of  $rb(p)$  on  $p^2(p)$  is more pronounced (see above).

STURTEVANT and TAN (1937) homologized  $pr(p)$  with  $bw(m)$ , especially because the double recessives  $v$   $pr(p)$  and  $or$   $pr(p)$  on one hand, and  $v$   $bw(m)$  and  $cn$   $bw(m)$  on the other give almost white eye colors. The optic



discs of *bw(m)* and *pr(p)* give rise in the transplantation experiments to identical eye colors. The four genes of this group behave differently if individuals carrying them are used as hosts for *or(p)*, *cn(m)*, *v(p)* and *v(m)* implants. The *pr(p)* and *bw(m)* hosts supply proportionately less *cn*<sup>+</sup> and more *v*<sup>+</sup> substance than *p*<sup>2</sup>(*p*) and *p*<sup>2</sup>(*m*) do. As implants, all four of these genes behave alike. In *pr(p)* and *p*<sup>2</sup>(*p*) hosts the + anlage develops a color intermediate between + and *pr(p)* and + and *p*<sup>2</sup>(*p*) respectively, but in *bw(m)* and *p*<sup>2</sup>(*m*) hosts the development is autonomous. This may be taken as evidence against the assumption that these various genes are homologous. From the facts stated above we are inclined to conclude that *pr(p)* is homologous to *bw(m)* and *p(p)* to *p(m)*.

g) *The se group* [*se(p)*, *se(m)*]. The colorations of the adult eyes produced by *se(p)* and *se(m)* are similar; no other genes have effects resembling those of the two genes just mentioned. The testicular envelope of *se(p)* and *se(m)* is pale, and the eyes become very much darker with age than they are at hatching. The double recessives *v se(p)* and *v se(m)* have an orange eye color in young flies.

If *se(p)* discs are implanted into *se(p)* hosts, or *se(m)* ones into *se(m)* hosts, the colors of the implants are alike. Likewise, the implantation of *se(p)* and *se(m)* into *v(p)* produces identical eye colors. Nevertheless, if discs carrying genes belonging to the *v* group are implanted into *se(p)*, the outcome is different from that observed if *se(m)* hosts are used. A difference in the outcome is observed also when *se(p)* and *se(m)* are implanted into hosts of the + group, or vice versa. Until more information on the nature of the eye pigments in the two species is available, the significance of these differences in the behavior of *se(p)* and *se(m)* is not clear. At present the assumption that *se(p)* is homologous to *se(m)* seems most likely to be true.

#### DISCUSSION

A list of the assumed homologies between the eye color genes of *D. pseudoobscura* and *D. melanogaster* is presented in table 4. Our results agree throughout with those of DONALD (1936) and STURTEVANT and TAN (1937). So far there is no evidence to show that any genes have been transferred from one chromosome to the other in these species; in other words, translocations seem to be very rare in the evolution process as compared with intra-chromosomal changes, such as inversions. As shown by DOBZHANSKY and TAN (1936) the differences between the gene arrangements in *D. pseudoobscura* and *D. miranda* are also due mainly to inversions, although translocations of short sections have been established in addition.

One difference between these two species in respect to eye color may be that the "wild type" *D. pseudoobscura* "normally" possesses the gene *cl(m)* in homozygous condition. In *D. melanogaster*, on the other hand, the

"wild type" allele of *cl(m)* is "normally" present. In addition, it appears that all *D. pseudoobscura* genes supply less *cn*<sup>+</sup> and *v*<sup>+</sup> substances than the homologous genes in *D. melanogaster*. Clot (m) acts like the *pseudoobscura* genes. + (m) and *Hn*<sup>+</sup>(m), for example, although they resemble *cl(m)* closely, differ from it in that they have more *v*<sup>+</sup> and *cn*<sup>+</sup> substances. Transplantation experiments with eye anlagen of the double recessives *v cl(m)* and *cn cl(m)* are under way. We expect more conclusive evidence from these experiments. The experiments with body fluid injection will

|           |   |       |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
|-----------|---|-------|------|------|------|-------|------|------|-------|--|---|--|--|--|------|--|------|------|
| $XL_P$    | <table><tr><td></td><td>W</td><td>RB</td><td></td><td>V</td><td></td></tr><tr><td></td><td></td><td>65.3</td><td></td><td>68.5</td><td>69.1</td></tr></table> |       |      |      |      |       |      | W    | RB    |  | V |  |  |  | 65.3 |  | 68.5 | 69.1 |
|           | W   | RB    |      | V    |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
|           |   | 65.3  |      | 68.5 | 69.1 |       |      |      |       |  |   |  |  |  |      |  |      |      |
| $X_M$     | W   | RB    | CM   | V    | G    | CAR   |      |      |       |  |   |  |  |  |      |  |      |      |
|           | 1.5   | 7.5   | 18.2 | 33.0 | 44.4 | 62.5  |      |      |       |  |   |  |  |  |      |  |      |      |
| $XR_P$    | <table><tr><td>MG</td><td>ST</td><td>SE</td></tr><tr><td>98.9</td><td>?</td><td>112.7</td></tr></table>   |       |      | MG   | ST   | SE    | 98.9 | ?    | 112.7 |  |   |  |  |  |      |  |      |      |
| MG        | ST  | SE    |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
| 98.9      | ?   | 112.7 |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
| $III_M$   | <table><tr><td>HN</td><td>SE</td><td>ST</td></tr><tr><td>23.0</td><td>26.0</td><td>44.0</td></tr></table>   |       |      | HN   | SE   | ST    | 23.0 | 26.0 | 44.0  |  |   |  |  |  |      |  |      |      |
| HN        | SE  | ST    |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
| 23.0      | 26.0  | 44.0  |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
| $II_P$    | <table><tr><td>P</td><td>CA</td><td>CN</td></tr><tr><td>57.9</td><td>60.2</td><td>82.0</td></tr></table>  |       |      | P    | CA   | CN    | 57.9 | 60.2 | 82.0  |  |   |  |  |  |      |  |      |      |
| P         | CA  | CN    |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
| 57.9      | 60.2  | 82.0  |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
| $III_R_M$ | <table><tr><td>P</td><td>CD</td><td>CA</td></tr><tr><td>48.0</td><td>74.7</td><td>100.7</td></tr></table>   |       |      | P    | CD   | CA    | 48.0 | 74.7 | 100.7 |  |   |  |  |  |      |  |      |      |
| P         | CD  | CA    |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
| 48.0      | 74.7  | 100.7 |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
| $III_P$   | <table><tr><td>OR</td><td>PR</td></tr><tr><td>0.0</td><td>49.9</td></tr></table>  |       | OR   | PR   | 0.0  | 49.9  |      |      |       |  |   |  |  |  |      |  |      |      |
| OR        | PR  |       |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
| 0.0       | 49.9  |       |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
| $II_R_M$  | <table><tr><td>CN</td><td>BW</td></tr><tr><td>57.5</td><td>104.5</td></tr></table>  |       | CN   | BW   | 57.5 | 104.5 |      |      |       |  |   |  |  |  |      |  |      |      |
| CN        | BW  |       |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |
| 57.5      | 104.5   |       |      |      |      |       |      |      |       |  |   |  |  |  |      |  |      |      |

TABLE 4

Homology of the eye color genes in *D. pseudoobscura* and *D. melanogaster* (see text).

make clear the relationship of the different substances in the different eye color groups. We may conclude from our experiments to date that the source of *cn*<sup>+</sup> and *v*<sup>+</sup> substances in *D. pseudoobscura* is essentially the same as in *D. melanogaster*.

Because in certain cases the implanted anlagen carrying genes of the *rb* group show a change to "wild type" as do *or(p)*, *cn(m)*, *v(p)* and *v(m)*, we conclude that there is a *rb* substance. The evidence that this new substance is not related to the *cn*<sup>+</sup> and *v*<sup>+</sup> substances is given (a) by the experiments of + (p) implanted into *rb(p)*, *or(p)* or *v(p)*; (b) by implants of *or(p)* and *v(p)* into *rb(p)*; (c) by the reciprocal experiments of (a) and (b); and (d) by

implants of  $pr(p)$  and  $p^2(p)$  into  $rb(p)$ ,  $or(p)$  or  $v(p)$ . The genes of the  $+$  group possess  $cn^+$  and  $v^+$ , but no  $rb$  substance. Orange ( $p$ ) and  $cn(m)$  have  $v^+$  and  $rb$ , but no  $cn^+$  substance. The genes of the  $pr$  group possess  $cn^+$  and  $v^+$ , but no  $rb$  substance. Ruby itself also has  $cn^+$  and  $v^+$ , but no  $rb$  substance. It is impossible to construct a step by step change from one of these three substances to another.

BEADLE and EPHRUSSI (1936) assumed originally that the presence of  $ca^+$  substance is the first step toward the formation of  $v^+$  substance, and that the production of  $v^+$  substance is a preliminary step toward  $cn^+$  substance. However, later transplantation experiments of EPHRUSSI and BEADLE (1936), and their experiments with body fluid (1937) have shown

|                | CN <sup>+</sup> | V <sup>+</sup> | RB | X |
|----------------|-----------------|----------------|----|---|
| + <sub>P</sub> | +               | +              | —  | — |
| ST             | +               | +              | +  | — |
| RB             | +               | +              | —  | + |
| OR             | —               | +              | +  | — |
| V              | —               | —              | +  | — |
| CA             | —?              | —?             | +  | + |
| PR             | +               | +              | —  | + |

TABLE 5

*The presence or absence of  $cn^+$ ,  $v^+$ ,  $rb$  substances and "X" in the different eye color groups (see text).*

that " $ca$ " possesses both  $cn^+$  and  $v^+$  substances. The results of our experiments show that a chain of reactions, in the sense that the formation of  $ca^+$  substance is the first step toward the formation of any other substance, is very unlikely. We are inclined to conclude that there is no  $ca^+$  substance at all present, but that in a few groups, of which " $ca$ " is an example, something, "X" is concerned, whose presence or absence prevents the optic discs of the  $+$  group from developing the "normal" eye color.

It is impossible at present to assume an " $se$ " substance. Whenever  $se(p)$  anlagen are implanted, an entirely new eye color type develops. We defer a discussion of the " $se$ " cases until experiments involving the second and the third instars shall have been finished. Two explanations are possible: (a) the anomalous behavior of " $se$ " optic discs in transplantation

may be the result of differences between host and implant in the period of development at which critical concentrations of the respective substances become available; (b) on the other hand, it is possible, and seems at present more probable, that in the different eye colors, entirely different substances and reactions are involved, giving rise through interactions to a new process.

+ (p) is not completely autonomous in *or*(p) and *v*(p), but develops a brighter eye color. This may be due rather to the different relationships of substances and reactions in these types, than to the presence in *or*(p) and *v*(p) of a special substance which changes the "wild type" eye color toward a brighter type.

Table 5 reviews the presence of substances in the different groups. The sign "+" indicates presence, the sign "-" absence. The question marks in the "ca" row for *cn*<sup>+</sup> and *v*<sup>+</sup> substances indicate that in certain experiments the presence of *cn*<sup>+</sup> and *v*<sup>+</sup> substances in "ca" is established. The reason that we did not record the *se* group is obvious from our statements above. We did not quote in table 5 the change of *pr*(p) and *p*<sup>2</sup>(p) implanted into *rb*(p), because the non-autonomous development resulted in a new eye color, which was that of neither the host nor the donor. For both latter cases also the statements above are valid.

#### ACKNOWLEDGMENTS

The authors are grateful to the members of the staff of the Wm. G. Kerckhoff Laboratories of the Biological Sciences for suggestions and criticism and to Miss E. Wallace for the drawings included in the tables.

#### SUMMARY

Optic discs can be successfully transplanted from one species of *Drosophila* to another. The transplanted discs give rise in the interspecific experiments to eyes which always develop autonomously in respect to the size and the color characteristic of its species.

The experiments show that, so far as eye color genes are concerned, no translocations have occurred in the two species since their divergence from a common ancestor.

In both species genes which belong to the same group behave similarly.

We assume that the following genes are homologous: *w*(p)-*w*(m); *cn*(p)-*cd*(m); *st*(p)-*st*(m); *rb*(p)-*g*(m); *v*(p)-*v*(m); *or*(p)-*cn*(m); *ca*(p)-*ca*(m); *pr*(p)-*bw*(m); *p*(p)-*p*(m); *se*(p)-*se*(m). The gene *mg*(p) has no homologous gene in *D. melanogaster*.

"Wild type" *D. pseudoobscura* is in its behavior homologous to *cl*(m). "Wild type" *D. pseudoobscura* and "wild type" *D. melanogaster* differ only quantitatively in the relationships to the various eye color genes.

Our data indicate the presence of at least one new substance, *rb* substance.

The existence of a *ca*<sup>+</sup> substance seems unlikely. Claret (*p*), *pr*(*p*), *p*<sup>2</sup>(*p*) and *rb*(*p*) prevent the development of "normal" eye color in implanted optic discs of the + group.

The assumption that a chain of substances is present, in such a way that one substance is necessary as a preliminary step to the formation of another substance, seems valid only for *cn*<sup>+</sup> and *v*<sup>+</sup> substances.

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GENETICS OF NATURAL POPULATIONS. I. CHROMOSOME  
VARIATION IN POPULATIONS OF *DROSOPHILA*  
*PSEUDOOBSCURA* INHABITING ISOLATED  
MOUNTAIN RANGES

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Received December 6, 1937

INTRODUCTION

IN RECENT years there appears to be a growing interest in the genetics of free-living populations, a subject hitherto almost untouched. Certain species of *Drosophila* prove to be very suitable for investigations in this field. TSCHETWERIKOFF (CHETVERIKOV), DUBININ and their collaborators in Russia, TIMOFEEFF-RESSOVSKY in Germany, GORDON in England, and STURTEVANT in this country have secured much valuable information which opens new vistas and raises a host of new problems (for a short review of the literature see DOBZHANSKY 1937). It has seemed to us that a comparison of the genetic constitutions of several free-living populations from the same general region, and yet isolated from each other, may be of interest. Samples of the populations of *Drosophila pseudoobscura* inhabiting island-like mountain forests in the Death Valley region of California and Nevada were collected. This region is especially favorable for our purposes, since it is as yet practically undisturbed by man's activities.

We wish to acknowledge our obligations to Professor A. H. STURTEVANT for his valuable advice, to Mr. H. D. CURRY whose help enabled us to collect material in certain rather inaccessible localities, and to Messrs. G. T. RUDKIN and EDWARD HELD for their assistance in conducting the experiments.

MATERIAL

The nature and source of the material are so important in the present investigation that we are forced to consider them more carefully than is customary in genetic accounts.

East of the rampart of the Sierra Nevada, in California and the adjacent part of the state of Nevada, there lies an extremely arid desert plateau. This plateau is strewn with mountain ranges and furrowed by deep valleys, extending mostly in the meridional direction (fig. 1). Some of the ranges lift their crests much above six thousand feet, and their upper reaches are covered by open forests consisting of piñon (*Pinus monophylla*), Juniperus, oak, and (on Charleston Peak and the Sierra Nevada), western yellow pine (*Pinus ponderosa*) and other conifers. The valleys intervening

between the ranges are barren except for a very xerophytic vegetation. The mountain forests may, consequently, be described as a series of islands surrounded by a sea of deserts. The contrast between the climatic and ecological conditions encountered on the mountain tops and in the valleys is exceedingly sharp.

The biology of *Drosophila pseudoobscura* in the natural state is very little known. According to the observations of DUDA (1924) and of STURTEVANT (unpublished), its relatives (*D. obscura*, *D. affinis* and other species) feed on the fermenting sap of bleeding trees, and the same is probably true for *D. pseudoobscura*. At any rate, the latter species is restricted to regions having some tree vegetation; occasionally it may be trapped a few thousand feet from the nearest trees, but it has never been found in terrain having only grass vegetation. In arid regions *D. pseudoobscura* is confined to mountains where forests can grow, and in general the denser the forest the more abundant is the fly population. To what extent populations inhabiting different mountain ranges exchange individuals it is very difficult to tell. During the warm season of the year the desert appears to be a formidable obstacle to the distribution of the fly, but an occasional transfer of a stray individual by wind or other agents is not excluded. At the very best, a migration of flies within a continuously forested region is easier than from one range to another across desert valleys. The population inhabiting each mountain range is largely, if not completely, isolated from others.

Samples of the population of *D. pseudoobscura* have been collected in eleven localities shown on the map (fig. 1). Each locality corresponds to a forested mountain range, and in the area studied there exist only two or three ranges where collecting has not been attempted. The entire collecting was accomplished within two months, from the middle of May to the middle of July, 1937; the genetic analysis of the samples has been carried on as nearly simultaneously as possible. The following technique has been adopted. Ten to twenty trap bottles with fermenting banana mush were exposed in each locality, the distance between the traps farthest apart being no less than a quarter and no more than one mile. The traps were left out for a few hours around sunset, and in some cases overnight. The flies that entered the traps were placed in vials with food and transported without delay to the laboratory. In the following presentation each sample of flies is referred to by the name of the locality (island forest) in which it was collected. It must be emphasized, however, that each sample comes from only a small fraction of the area of a given forest; the possibility that the populations inhabiting different parts of the same forest may be different is by no means excluded.

## THE GENE ARRANGEMENT IN THE THIRD CHROMOSOME

Strains of *D. pseudoobscura* coming from the same or from different localities are frequently unlike in the gene arrangement. The third chromosome proves to be more variable in this respect than the rest; seventeen arrangements related to each other as single or multiple inversions have

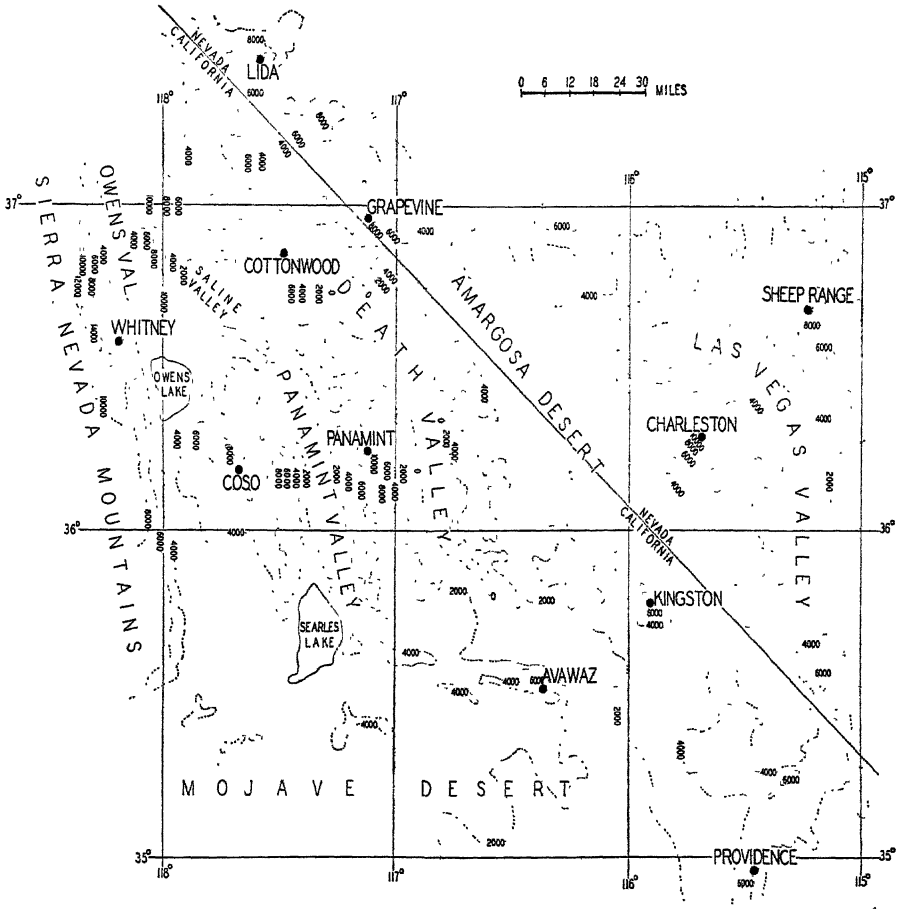


FIGURE 1. Map showing localities from which collections were made. (Note: Avawaz should be spelled Awavaz as in text.)

been recorded (STURTEVANT and DOBZHANSKY 1936a; DOBZHANSKY and STURTEVANT 1938). Populations inhabiting the mountain ranges in the Death Valley region also proved to be variable in the gene arrangement, and the variability observed is of a kind that permits certain inferences as to the relation of these populations to each other.

The gene arrangement in a given individual can be determined by



inspection of the chromosomes in the cells of the salivary glands. In structurally homozygous individuals the distribution of the stainable discs in the chromosomes reflects the arrangement involved; if two homologous chromosomes differing in the gene arrangement are present, a characteristic pairing configuration results. For descriptions of each of the seventeen arrangements the paper by DOBZHANSKY and STURTEVANT (1938) may be consulted.

Since females caught outdoors are usually fertilized already, they were placed in individual cultures and allowed to produce offspring. Wild males were crossed to females homozygous for the third chromosome recessives orange (*or*) and purple (*pr*), and having the standard gene arrangement in the third and in all other chromosomes. From the offspring of each wild female a single larva was taken, and a temporary acetocarmine preparation of its salivary glands was made. Such a larva carries one maternal and one paternal third chromosome, and therefore an examination of its nuclei furnishes the information on the gene arrangement in two chromosomes present in the wild population in question. From each male culture seven larvae were taken, and one salivary gland from each larva was fixed and stained in acetocarmine. If the male parent contained two third chromosomes similar in the gene arrangement, all seven larvae show identical chromosome configurations; if two different chromosomes were present, the offspring fall into two equally numerous classes, and the probability is sufficiently great that among seven larvae both classes are represented (only one out of sixty-four heterozygotes is thus not detected). The third chromosomes were carefully examined in every preparation, the rest receiving only a cursory inspection.

Four previously known gene arrangements have been found in populations coming from the localities shown in figure 1. These have been designated as "Standard," "Arrowhead," "Chiricahua," and "Mammoth" respectively (DOBZHANSKY and STURTEVANT 1928). In the offspring of a female collected on Kingston Peak there was found a single larva showing a fifth, and previously unknown, arrangement in the third chromosome. The culture was lost before the new arrangement could be studied in detail. Table 1 shows the frequencies of the four arrangements in the eleven populations; each observed frequency is accompanied by its probable error. The total numbers of the chromosomes examined are given in the right-most column.

An examination of table 1 shows that the Arrowhead, Chiricahua, and Standard arrangements are present in every population, Arrowhead being much the commonest. The Mammoth arrangement was found in only two localities, a single chromosome in each case. Its frequency is therefore negligible. Nevertheless, some of the populations are significantly different

from others in the relative frequency of the different arrangements. Only three localities, namely Panamint, Awavaz, and Charleston, are inhabited by populations that seem to be identical in this respect. The Coso population is slightly but significantly different from the preceding three. The remaining six populations differ significantly from each other as well as from the four mentioned above, although the sample from Mt. Whitney is too small to be much relied upon.

TABLE I  
*Frequencies (in percent) of the four gene arrangements encountered in the third chromosome.*

| MOUNTAIN RANGE       | ARROWHEAD    | CHIRICAHUA   | STANDARD     | MAM-MOTH | CHROMOSOMES TESTED |
|----------------------|--------------|--------------|--------------|----------|--------------------|
| Lida (Mt. Magruder)  | 76.80 ± 1.80 | 6.00 ± 1.01  | 16.80 ± 1.59 | 0.40     | 250                |
| Mt. Whitney          | 69.57 ± 4.57 | 8.70 ± 2.73  | 21.73 ± 4.10 | —        | 46                 |
| Coso                 | 72.27 ± 1.89 | 14.45 ± 1.48 | 13.28 ± 1.43 | —        | 256                |
| Cottonwood           | 51.20 ± 2.13 | 9.60 ± 1.25  | 38.80 ± 2.08 | 0.40     | 250                |
| Grapevine (Funeral)  | 50.86 ± 2.23 | 18.70 ± 1.73 | 30.43 ± 2.04 | —        | 230                |
| Panamint (Telescope) | 67.41 ± 2.11 | 18.75 ± 1.75 | 13.83 ± 1.56 | —        | 224                |
| Awavaz               | 62.20 ± 3.61 | 19.51 ± 2.95 | 18.29 ± 2.88 | —        | 82                 |
| Kingston             | 64.08 ± 2.25 | 5.34 ± 1.06  | 30.58 ± 2.17 | —        | 206                |
| Charleston           | 68.75 ± 1.96 | 19.14 ± 1.66 | 12.11 ± 1.38 | —        | 256                |
| Sheep Range          | 88.11 ± 1.53 | 1.98 ± 0.64  | 9.90 ± 1.42  | —        | 202                |
| Providence           | 82.00 ± 1.50 | 10.00 ± 1.17 | 8.00 ± 1.06  | —        | 300                |

A more detailed analysis of the data presented in table 1 brings to light some additional facts. It must be borne in mind that each of the three arrangements commonly found in the geographical region now under study is encountered also beyond its confines (DOBZHANSKY and STURTEVANT 1928). The Arrowhead arrangement is present throughout the distribution area of the species, except in southern Mexico. The populations inhabiting New Mexico, southern Colorado, northern Arizona, and most of southern Utah show a hundred percent frequency of Arrowhead; westward and north-westward from there the frequency of Arrowhead declines, and reaches a minimum on the Pacific Coast. Standard shows the opposite regularity: its frequency is highest on the Pacific Coast, and decreases as one proceeds eastward, reaching zero just east of the Death Valley region. Finally, Chiricahua is a southern type, very common in Mexico, and the northernmost locality where it has been recorded is in the Toyabe Range, Nevada, lying less than a hundred miles north of Lida. Are, then, the differences observed between the relatively closely adjacent localities in the region studied by us merely an expression of the general geographic trends in the distributions of the gene arrangements? If that were so, one might expect the frequency of Standard to be greatest in the western or

north-western localities, and to decrease as one proceeds eastward. A similar gradient in the direction south-north would be expected for Chiricahua, and an east-west gradient for Arrowhead. Indeed, gradients of this kind have been observed by many students of geographical variation in all sorts of animals and plants (for example, SUMNER 1929).

An examination of table 1 in conjunction with figure 1 reveals however no definite gradients. The greatest frequency of Standard obtains in the centrally located Cottonwood mountains, and decreases westward as well as eastward. Chiricahua is more frequent in the center (Awavaz, Panamint, Charleston) than it is in the south (Providence). Arrowhead is most frequent on the fringes of our region, and least frequent in the center. Not only is there no regularity if our region is treated as a part of the general species area, but there are no definite trends within the region itself. Mountain ranges that lie far apart may be more similar than adjacent ones. Thus, Charleston and Awavaz are alike, while Kingston which is located almost exactly half way between the two is distinctly different from either. The frequency of the Chiricahua arrangement is very low on Kingston Peak and Sheep Range, and high on Charleston which lies between them. Panamint is similar to the rather remote Awavaz and Charleston mountains, and very different from the Cottonwood mountains which represent merely a northern extension of the Panamint. Sheep Range and Providence are more similar to each other and to the remote Lida than to the intervening Charleston and Kingston Peaks. In short, the variation seems to be random. The possible significance of this fact is discussed below.

The data on the gene arrangement in the third chromosome were collected in such a way that in every case two chromosomes present in a given individual were examined. This permits us to study the relative frequencies in natural populations of individuals homozygous and heterozygous for the different gene arrangements. Let the gametic frequencies of the Arrowhead, Chiricahua, and Standard arrangements be  $a$ ,  $c$ , and  $s$  respectively ( $a + c + s = 1$ ). If mating is random, and the viabilities of the structural homo- and heterozygotes are alike, the expected frequencies of the different types are as follows:

Homogyzous Arrowhead— $a^2$

Homogyzous Chiricahua— $c^2$

Homogyzous Standard— $s^2$

Heterozygous Arrowhead/Chiricahua— $2ac$

Heterozygous Arrowhead/Standard— $2as$

Heterozygous Chiricahua/Standard— $2cs$

A comparison of the observed and expected frequencies is presented in table 2. In general, the two sets of values agree very well, attesting that

TABLE 2

*The observed and expected frequencies of the inversion homozygotes and heterozygotes in various populations.*

| LOCALITY    |      | HOMOZYGOTES |            |          | HETEROZYGOTES |           |            |
|-------------|------|-------------|------------|----------|---------------|-----------|------------|
|             |      | ARROWHEAD   | CHIRICAHUA | STANDARD | ARROWHEAD     | ARROWHEAD | CHIRICAHUA |
|             |      |             |            |          | CHIRICAHUA    | STANDARD  | STANDARD   |
| Lida        | Obs. | 74          | —          | 6        | 14            | 29        | 1          |
|             | Exp. | 73.75       | 0.45       | 3.5      | 11.5          | 32.2      | 2.5        |
| Coso        | Obs. | 70          | 2          | 6        | 28            | 17        | 5          |
|             | Exp. | 66.85       | 2.7        | 2.25     | 26.7          | 24.6      | 5.0        |
| Cottonwood  | Obs. | 34          | 2          | 21       | 13            | 47        | 7          |
|             | Exp. | 32.75       | 1.5        | 18.9     | 12.3          | 49.6      | 9.3        |
| Grapevine   | Obs. | 27          | 3          | 12       | 27            | 36        | 10         |
|             | Exp. | 29.75       | 4.0        | 10.65    | 21.9          | 35.5      | 13.1       |
| Panamint    | Obs. | 49          | 3          | 3        | 32            | 21        | 4          |
|             | Exp. | 50.8        | 3.9        | 2.1      | 28.2          | 20.9      | 5.8        |
| Kingston    | Obs. | 45          | 1          | 12       | 6             | 36        | 3          |
|             | Exp. | 42.3        | 0.3        | 9.6      | 7.1           | 40.4      | 3.4        |
| Charleston  | Obs. | 62          | 7          | 5        | 33            | 19        | 2          |
|             | Exp. | 60.5        | 4.7        | 1.9      | 33.7          | 21.3      | 5.9        |
| Sheep Range | Obs. | 77          | —          | —        | 4             | 20        | —          |
|             | Exp. | 78.4        | 0.04       | 0.99     | 3.5           | 17.6      | 0.04       |
| Providence  | Obs. | 102         | 3          | 2        | 22            | 20        | 1          |
|             | Exp. | 100.9       | 1.5        | 1.0      | 24.6          | 19.7      | 2.4        |

the above assumptions of the randomness of mating and equal viability are correct. A more detailed examination of table 2 shows however that the observed figures for homozygotes are somewhat more frequently higher than lower than the expected ones, and the reverse is true for the heterozygotes. The differences are in no case statistically significant. This small discrepancy, if real, may have two explanations. First of all, a small fraction of the heterozygotes might have been misclassified as homozygotes (see above). Secondly, the fly samples from each locality were collected in a number of trap bottles placed at some distance from each other. If the flies spend their life in the immediate vicinity of the place where they were born, the mating may not be absolutely random, and a certain amount of inbreeding may be involved. This latter possibility is theoretically very interesting, but no conclusions can be drawn from the data now available. What is demonstrated is merely that the natural populations are not segregated into non-interbreeding sections each of which has a given gene arrangement.

#### THE "SEX-RATIO"

Certain males of *D. pseudoobscura* produce, when crossed to females of any genetic constitution, mostly or only daughters and few or no sons.

This peculiar "sex-ratio" condition is known to be inherited through the X chromosome, and to be associated with an inversion in the right limb of the latter, the association apparently being invariable. The "sex-ratio" is often encountered in wild populations of *D. pseudoobscura*, but the frequency is higher in the southern than in the northern part of the specific area (STURTEVANT and DOBZHANSKY 1936b). It proved to be rather common in most of the populations from the Death Valley region.

To detect the presence or absence of the "sex-ratio," wild males or single sons of wild females were crossed in individual cultures to two or three or *pr* females. In the F<sub>1</sub> generation some of the cultures produced few or no sons, indicating that the father carried the "sex-ratio" in its X chromosome. Complete counts are unnecessary to distinguish the "sex-ratio" from the normal cultures, because in the former only very few or no males are found. Since the salivary gland chromosomes of the F<sub>1</sub> larvae were studied in order to determine the gene arrangement in the third and the other chromosomes, some "sex-ratio" cultures were in fact detected before any adult flies appeared in them. Nevertheless, no special attention was paid to the presence or absence of the "sex-ratio" inversion in the right limb of the X chromosome in these cultures, so that the statistical data of the frequency of the "sex-ratio" presented in table 3 are based on the genetical and not on cytological determinations.

TABLE 3  
*Frequency (in percent) of the "sex-ratio" condition in the X chromosome.*

| LOCALITY    | ELEVATION<br>(FEET) | DATE OF<br>COLLECTING | SEX-RATIO | CHROMOSOMES<br>TESTED |
|-------------|---------------------|-----------------------|-----------|-----------------------|
| Awavaz      | 5000                | May 22                | 23.08     | 26                    |
| Providence  | 5000?               | May 7, 8              | 16.06     | 137                   |
| Sheep Range | 6000                | June 5                | 11.57     | 121                   |
| Kingston    | 4800                | May 21                | 11.38     | 123                   |
| Cottonwood  | 7800                | June 23, 24           | 10.66     | 122                   |
| Lida        | 8000                | June 15, 17           | 9.09      | 66                    |
| Grapevine   | 7500                | June 22               | 8.33      | 72                    |
| Panamint    | 8300                | May 20                | 8.09      | 136                   |
| Charleston  | 8200                | June 4                | 6.72      | 119                   |
| Coso        | 6500                | July 14, 15           | 4.70      | 149                   |

From 4.7 percent (in Coso) to 23.1 percent (in Awavaz mountains) of the X chromosomes were found to carry the "sex-ratio." Table 3 shows also the approximate elevation above sea level of the localities in which the collecting was done, and the date of collecting. There seems to be a weak negative correlation between the elevation and the frequency of the "sex-ratio"; the existence of such a correlation has been suspected on the basis of another set of data (STURTEVANT and DOBZHANSKY 1936b).

Taken at their face value, the figures in table 3 suggest that the frequency of the "sex-ratio" in localities of equal elevation diminishes as the season advances, but the reality of this relationship is certainly far from established.

#### CHROMOSOMAL VARIATION IN RACE B

All of the above data concern race A of *D. pseudoobscura*. Race B of the same species has been found only in two out of the eleven localities shown in figure 1, namely on Mt. Whitney and in Coso mountains. On Mt. Whitney more than three times as many race B as race A flies came into the traps, while in Coso 3 race B individuals were encountered together with 149 of race A. The geographical distribution of race B lies west and north-west from the Death Valley region, Coso mountains being in fact the extreme south-eastern locality where this race has been thus far encountered.

The gene arrangement was studied in 74 race B third chromosomes from Mt. Whitney and in 6 chromosomes from Coso. On Mt. Whitney 15 chromosomes, or 20.3 percent, showed the Standard, and 1, or 1.35 percent, the Klamath arrangement. The remaining 58, or 78.4 percent, of the chromosomes had a gene arrangement which has not been encountered previously. This new arrangement, designated as "Whitney," differs in a single inversion from Klamath and in two inversions from the Standard (Klamath and Standard differ in a single inversion). The distal end of the Whitney inversion lies proximally from the distal end of the Klamath one, and the proximal end of Whitney lies nearer to the base of the chromosome (section 69A) than in Klamath. The gene arrangement in Whitney is therefore on the whole more similar to that in the Standard than to that in Klamath. Out of the six chromosomes from Coso, three had the Whitney and three the Standard arrangement.

The discovery of a new arrangement in the third chromosome of *D. pseudoobscura* (the eighteenth in the species) is in itself not surprising. What is more unexpected is that the new arrangement has been found to be so frequent on Mt. Whitney, on the eastern slope of the Sierra Nevada, while it has not been encountered at all among race B individuals from the Sequoia National Park (Giant Forest and Lodgepole Camp), from which samples of flies had been previously studied (DOBZHANSKY and STURTEVANT 1938). The Giant Forest and Lodgepole Camp localities lie less than a hundred miles from Mt. Whitney, on the eastern slope of the Sierra Nevada. On the contrary, two other gene arrangements known from Sequoia Park have not been found on Mt. Whitney. This suggests that populations of *D. pseudoobscura* are very local, and that few migrants pass from one locality to the other. One must note however that the Giant Forest and Mt. Whitney localities are separated by the alpine desert of

the highest part of the Sierra Nevada, which may constitute a natural barrier for the distribution of *D. pseudoobscura*.

#### DISCUSSION

It has been shown above that three distinct gene arrangements in the third chromosome, namely Standard, Arrowhead, and Chiricahua, are encountered in every one of the eleven populations studied. The relative frequencies of these arrangements, however, vary within rather wide limits; there is no geographical trend or regularity in these variations, so that populations from adjacent localities may be more different from each other than populations from remote ones. In order to evaluate properly the significance of these observations one must recall certain other facts discussed in detail by DOBZHANSKY and STURTEVANT (1938). The gene arrangements known in the third chromosome of *D. pseudoobscura* are members of a single phylogenetic series. Some arrangements differ from others in a single inversion, while others are related only through a chain of discrete intermediate forms, each link in the chain representing a single inversion step. Thus, Arrowhead and Standard differ in a single inversion, while it takes three inversions to pass from Standard to Chiricahua, four to derive Chiricahua from Arrowhead, three to derive Mammoth from Standard, and two to get Mammoth from Chiricahua, or vice versa. It is significant that in the mountains of the Death Valley region we find only the gene arrangements just named, and do not find the intermediates between them (which, however, are encountered in other, more or less remote, geographical localities). This means that the variety of the gene arrangements now observed in the Death Valley region has arisen elsewhere, or at any rate that its origin has taken place at a certain more or less remote time. Only Standard and Arrowhead may be conceived to transform into each other by "mutation," although we not only lack evidence that they do so regularly, but even have reasons to doubt that this is the case.

What are, then, the driving causes of the historical process which has resulted in the differentiation of the populations inhabiting separate mountain ranges? Since the differences observed are merely quantitative, and since, taken as a whole, the Death Valley populations are intermediate between populations living in the surrounding territories, the possibility of migration as one of the causes must be considered. Arrowhead is more frequent to the east and less frequent to the west of the Death Valley region than anywhere in that region itself. For Standard the relations are the reverse of those for Arrowhead, while Chiricahua is most frequent to the south of Death Valley. If the rate of exchange of individuals between populations inhabiting parts of the distribution area of the species is great

enough, a chain of populations intermediate between the extremes will result. By "great enough" we mean a migration rate sufficient to outweigh other causes leading toward diversification of local populations. The populations inhabiting the Death Valley region form, however, no geographical chains linking together the populations of the surrounding country. We have seen that the characteristics of a population inhabiting a given mountain range are independent of those of the populations in the neighboring mountains. Each population seems to be sufficiently isolated from the others, and migration, if it occurs at all, is not a factor of paramount importance in determining the characteristics of a colony.

The possibility that the gene arrangement may not be indifferent for the viability of its carrier, and that selection has caused one or the other arrangement to be frequent on a given mountain range, must be considered. The localities where collecting was done differ in altitude, climate, flora, and other conditions. The frequency of "sex-ratio" shows indeed a suggestive correlation with the altitude of the locality. The "sex-ratio" cannot, however, be likened to the variations in the gene arrangement in the third chromosome; while the former produces an easily discernible effect, namely unisexual progenies, the latter do not seem to be correlated with any physiological or morphological characteristics of their carriers. There is also no trace of a correlation between the frequency of any gene arrangement in a given locality and any peculiarities of the latter. Since, furthermore, every one of the three gene arrangements is present in every locality, and presumably has been indigenous there for countless generations, the supposition that these chromosome structures are subject to selection is extremely improbable, although, by the very nature of things, it cannot be completely excluded.

By far the most probable explanation of the observed differences between populations of the separate mountain ranges is that the frequency of a gene or a chromosome structure is subject to random fluctuations. WRIGHT (1931, and other works) has shown that, theoretically, such fluctuations must occur in populations whose size is not infinitely large, and that the smaller the population the greater is the amplitude of the fluctuations in a given time interval. In a species segregated in numerous colonies this process will sooner or later lead to a differentiation, provided only that its intercolonial migration rate is not great enough to cancel the effects of the fluctuations. The mountain ranges of the Death Valley region, isolated from each other by the intervening deserts, furnish a set of conditions that might favor such a differentiation. Suppose, for example, that this region was inhabited originally by a homogeneous population in which the frequencies of the three commonest gene arrangements were like those now found in Panamint, Awavaz, and Charleston mountains



(fig. 1 and table 1). A segregation of this population into colonies restricted each to an isolated mountain forest might have resulted in the course of time in a diversification. In some populations, like those inhabiting the three mountain ranges just named, the original frequencies have been retained. Other populations have deviated to a smaller or greater extent from the original condition. But there is not, nor is there expected to be, any geographical trend or regularity in those fluctuations. Adjacent mountains are as likely to retain as they are to lose the similarity in the composition of their populations.

The factor that is decisive for an evaluation of the above hypothesis is how large is the effective size of the breeding population in the colonies of the flies inhabiting each mountain range? The greater the size, the shorter the time interval during which this differentiation may be supposed to have taken place, the less probable is the hypothesis, and vice versa. We hope to be able to present some information bearing on this problem at a later date.

#### SUMMARY

1. *Drosophila pseudoobscura* inhabits mountain forests in the Death Valley region, but not the intervening deserts. Samples of the fly populations were collected in eleven localities (fig. 1).
2. Three different gene arrangements in the third chromosome are present in every population studied. The difference between these arrangements is due to inversion of chromosome sections.
3. Inversion heterozygotes and homozygotes are present in every population in theoretically predictable proportions (table 2).
4. The relative frequency of each arrangement varies from locality to locality (table 1). Only three out of the eleven localities are inhabited by populations that seem to be identical with respect to the relative frequencies of the gene arrangements.
5. Populations inhabiting adjacent localities are no more likely to be similar than populations from remote ones.
6. It is concluded that the rate of migration of flies between localities is small. The genetic compositions of the populations inhabiting different localities may drift apart in the course of time.
7. A certain proportion of the X chromosomes carry the "sex-ratio" condition (table 3). There is a slight correlation between the frequency of the "sex-ratio" and the elevation of the locality above sea level.

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# SIZE INHERITANCE AND GEOMETRIC GROWTH PROCESSES IN THE TOMATO FRUIT

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Received December 29, 1937

## INTRODUCTION

THE literature on size inheritance contains but few references to what seems to be an important clue to its study, namely, a frequent tendency for  $F_1$  and  $F_2$  hybrids to approach the geometric mean of the parent sizes (GROTH 1914, 1915). It is almost universally assumed and taught that the ideal hybrid will "blend" in size to the arithmetic mean. The reasons for the comparative neglect of geometric relationships seem to be mainly that the studies of size inheritance hitherto made have been with few exceptions confined to crosses between parents so similar in size that arithmetic and geometric means are not easily distinguished. Such confusion is but natural, since size fluctuates widely under environmental influences, and is often confusingly increased over its true value by heterosis.

The classic data are in fact mainly drawn from crosses where the parent races or species differed by not more than 2, 3 or 4 to 1. Approximately such a ratio characterizes the parental differences in the bulk of the published crosses involving linear measurements or proportions, surface areas, volumes or weights of the body as a whole or of some part or organ. Witness the many studies on rodents and fowls among animals and the even more numerous similar observations on the inheritance of size of flowers, seeds, fruits, leaves, stems or roots of both cultivated and wild plants.

Much greater size differences than these are obtainable in crossable parents in selected cases. For instance artificial selection has produced an extremely large size range in fruits of apples, peppers and tomatoes, in tubers of potatoes, and a striking contrast in body size between bantam and Asiatic breeds of fowls, etc. Hybrids between such extremes generally show not blending but "dominance of small size," or inheritance by some geometric rule.

The tomato fruit provides especially favorable material for size study, since there are enormous differences between the small one gram fruits of "Red Currant" (*Lycopersicon pimpinellifolium*) and the comparatively gigantic 100 to 400 gram fruits of some commercial varieties of *L. esculentum*. During the last twelve years we have accumulated a vast amount of size data, and from them evolved a theory of size inheritance (MACARTHUR 1935, BUTLER 1937), here applied in the hope that it will clarify

TABLE I  
Mean fruit weight in grams of the different tomato crosses.

| CROSS NUMBER                              | LARGER PARENT     | SMALLER PARENT      | NO. OF F <sub>2</sub> PLANTS | LARGE P <sub>1</sub> | SMALL P <sub>1</sub> | F <sub>1</sub> | F <sub>2</sub> | GEOMETRIC MEAN | ARITHMETIC MEAN |
|---|-------------------|---------------------|------------------------------|----------------------|----------------------|----------------|----------------|----------------|-----------------|
| <i>Parents Differing Greatly in Size</i>  |                   |                     |                              |                      |                      |                |                |                |                 |
| 449                                       | Yellow Pear       | X Red Currant       | 84                           | 12.4                 | 1.1                  | 4.2            | 4.2            | 3.7            | 6.7             |
| 3413                                      | Large Pear        | X Red Currant       | 210                          | 54.1                 | 1.1                  | 7.4            | 6.4            | 7.4            | 27.6            |
| 25A                                       | Grape Cluster     | X Red Currant       | 75                           | 55.8                 | 1.1                  | 7.9            | 8.0            | 7.6            | 28.5            |
| 3373                                      | 902 selection     | X Red Currant       | 914                          | 50.2                 | 1.1                  | 7.2            | 7.3            | 7.6            | 28.6            |
| 34T                                       | Putman's Forked   | X Red Currant       | 124                          | 57.0                 | 1.1                  | 7.1            | 7.5            | 7.7            | 29.0            |
| 3316                                      | 745 selection     | X Red Currant       | 650                          | 57.5                 | 1.1                  | 7.4            | 7.8            | 7.7            | 29.3            |
| 452                                       | Honor Bright      | X Red Currant       | 143                          | 150.0                | 1.1                  | 9.4            | 6.9            | 12.3           | 75.5            |
| 441                                       | Golden Queen      | X Red Currant       | 136                          | 152.4                | 1.1                  | 10.1           | 10.2           | 12.3           | 76.7            |
| 3210                                      | Tangerine         | X Red Currant       | 720                          | 173.6                | 1.1                  | 8.3            | 9.5            | 13.2           | 87.3            |
| 414                                       | Albino            | X Red Currant       | 219                          | 312.0                | 1.1                  | 12.6           | 12.3           | 17.5           | 156.5           |
| 3415                                      | Tangerine         | X r y f H selection | 140                          | 173.6                | 5.0                  | 38.1           | 50.0           | 29.4           | 89.3            |
| 729                                       | p o r y selection | X Burbank Pres.     | 58                           | 30.0                 | 5.1                  | 14.0           | 15.8           | 13.5           | 20.6            |
| 901                                       | l c u h selection | X Burbank Pres.     | 238                          | 55.0                 | 5.1                  | 21.0           | 22.3           | 16.8           | 39.0            |
| 721                                       | Devon Surprise    | X Burbank Pres.     | 66                           | 58.0                 | 5.1                  | 23.0           | 22.9           | 17.2           | 32.5            |
| 403                                       | Dwarf Aristocrat  | X Yellow Pear       | 222                          | 112.6                | 12.4                 | 35.5           | 41.9           | 37.4           | 62.5            |
| 445                                       | McMullen Pink     | X Yellow Pear       | 286                          | 148.8                | 12.4                 | 44.0           | 45.2           | 43.0           | 80.6            |
| 451                                       | Honor Bright      | X Yellow Pear       | 136                          | 150.0                | 12.4                 | 47.5           | 46.1           | 43.3           | 81.2            |
| <i>Parents Differing Slightly in Size</i> |                   |                     |                              |                      |                      |                |                |                |                 |
| 3409                                      | r y f H selection | X Red Currant       | 86                           | 5.0                  | 1.1                  | 3.3            | 3.4            | 2.5            | 3.0             |
| 405                                       | Peach             | X Yellow Pear       | 495                          | 42.6                 | 12.4                 | 23.1           | 26.2           | 23.0           | 27.5            |
| 410                                       | Honor Bright      | X White Apple       | 183                          | 150.0                | 39.1                 | 49.9           | 57.2           | 76.5           | 94.5            |
| 401                                       | Dwarf Aristocrat  | X Peach             | 163                          | 112.4                | 42.6                 | 67.1           | 73.3           | 69.5           | 77.5            |
| 450                                       | Honor Bright      | X Peach             | 390                          | 150.0                | 42.6                 | 68.1           | 61.6           | 80.0           | 96.3            |
| 434                                       | Dwarf Aristocrat  | X Grape Cluster     | 90                           | 112.4                | 55.8                 | 73.3           | 81.1           | 79.0           | 84.1            |
| 3209                                      | Tangerine         | X 902 selection     | 534                          | 173.6                | 56.2                 | 112.3          | 86.3           | 99.0           | 114.9           |
| 3201                                      | Tangerine         | X R. N. H.          | 350                          | 173.7                | 68.0                 | 91.2           | 81.1           | 109.0          | 120.8           |
| 409                                       | Albino            | X Dw. Aristocrat    | 216                          | 312.0                | 112.4                | 137.9          | 160.4          | 188.0          | 212.2           |
| 438                                       | Albino            | X Honor Bright      | 80                           | 312.0                | 150.0                | 160.0          | 151.3          | 217.0          | 231.0           |

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some aspects of the problem and also suggest other avenues of research. As a good deal of this material is embodied in the thesis to be published later by the junior author, this paper will be confined to a statement of the theory with a modicum of supporting data. The observations will be presented first and will treat the subject from four different angles.

#### I. THE INHERITANCE OF FRUIT WEIGHT

There is a large amount of variation between the weights of fruits even on the same plant, but, as will be shown in a later paper, each variety or inbred line has its own average inherited weight. By crossing lines of different fruit weights we can see how the various contrasting fruit sizes are inherited. The average fruit weight was determined by weighing ten fruits from each plant, a certain amount of selection being exercised to avoid using obviously atypical fruits. These abnormal fruits were of two types, undersized ones due to faulty pollination or lop-sided growth, and those that were oversized owing to an increase in the locule number.

The crosses presented (table 1) are divided into two categories, first the cases where the two parents differ greatly in fruit size, and second, those in which the parents differ only slightly. It will be noticed that the resulting hybrids cover the whole range of possible behaviors, illustrating at the one extreme the geometric mean and at the other extreme typical blending. Closer scrutiny of this table will show that the cases that conform most closely to the arithmetic mean of the two parents are crosses in which the parents did not differ greatly in fruit size (small by small, medium by medium, or large by large). It will also be noted that only one  $F_1$  weight is given; it was found that even when the parental size contrast was greatest the reciprocal crosses never differed significantly in  $F_1$  fruit weight.

The main purpose of the table is to bring out the close agreement in both the interspecific and intraspecific crosses between the observed  $F_1$  and  $F_2$  means and the calculated geometric means, and the poor agreement with the arithmetic means. The greater the parental contrasts, the larger become the discrepancies between arithmetic and geometric values and the more the  $F_1$  and  $F_2$  values favor the geometric mean.

That a similar geometric relationship applies equally well in the case of backcrosses is shown in table 2.

Analyzed from this point of view numerous crosses and "grading up" backcrosses in other species show a similar behavior. This is illustrated in SAUNDER'S and MACOUN'S breeding project with the wild Siberian crab; by large commercial varieties of apples (CHIPMAN 1933); in peppers (DALE 1929); and in  $F_1$  hybrids of bantam fowls by large Asiatic breeds (JULL and QUINN 1931).

In these varied materials the approach of the  $F_1$ ,  $F_2$  and backcross sizes to the geometric means may be taken to be the general rule, the seeming approach to the arithmetic mean being really limited to those special cases in which parental differences are comparatively small. Since the latter happen to be most numerous in the genetic literature they have come to be considered typical, possibly obscuring thereby an essential feature of size inheritance.

TABLE 2

*Mean fruit weights in grams of two tomato crosses and their respective backcrosses.*

|                           | $P_1$           | B.C.           | $F_1, F_2$     | B.C.           | $P_1$           |
|---------------------------|-----------------|----------------|----------------|----------------|-----------------|
| Red Currant×Tangerine     | $1.12 \pm .005$ | $3.14 \pm .09$ | $9.03 \pm .13$ | $32.2 \pm .78$ | $173.6 \pm 3.6$ |
| Geometric mean            |                 | 3.19           | 13.8           | 39.5           |                 |
| Arithmetic mean           |                 | 5.08           | 87.3           | 91.3           |                 |
| Red Currant×902 selection | $1.12 \pm .005$ | $2.5 \pm .15$  | $7.41 \pm .10$ | $19.8 \pm .95$ | $56.2 \pm .05$  |
| Geometric mean            |                 | 2.9            | 7.9            | 20.4           |                 |
| Arithmetic mean           |                 | 4.27           | 28.6           | 31.8           |                 |

## 2. THE $F_2$ DISTRIBUTION OF FRUIT WEIGHTS

A prime essential for the study of problems of inheritance of size and other important features of organization is the merging of the physiological, developmental and genetic points of view. The problem thus becomes primarily one of determining the physiology of gene effects during the development of such quantitative characters.

In the recent literature there has been a tendency to attack the problem from the standpoint of gene action and to subject the older view of blending inheritance to considerable criticism. KAPTEYN (1916) long ago pointed out, with numerous appropriate biological examples, that causes depending on size produce proportional or positively skew distributions, instead of normal frequency curves. RASMUSSEN'S (1933) theory of genic interaction assumes that the total effect of the genotype is not determined by the direct simple addition of the effects of quantity genes, but that they interact with one another in such a way that the cumulative effect of all the genes is less than the sum of their individual effects; the net result is inevitably a negatively skew curve. POWERS (1936) in his work on barley actually obtained data directly opposing this view and concluded that the cumulative effect of a number of genes was greater than the sum of their individual effects. Recently SINNOTT (1937) has shown that his  $F_2$  frequency distributions in summer squashes are positively skewed and has interpreted this to indicate that the effects of the size genes are geometrically cumulative.

The form of the  $F_2$  distribution is thus often used as an index of the type of inheritance and the mode of operation of the size genes; for these purposes data are given from two  $F_2$  populations, each consisting of more than a thousand plants. In both cases the curves (fig. 1) are positively skew, and on the basis of such large numbers this skewness is significant. Since in cases of blending inheritance a normal curve is always attained

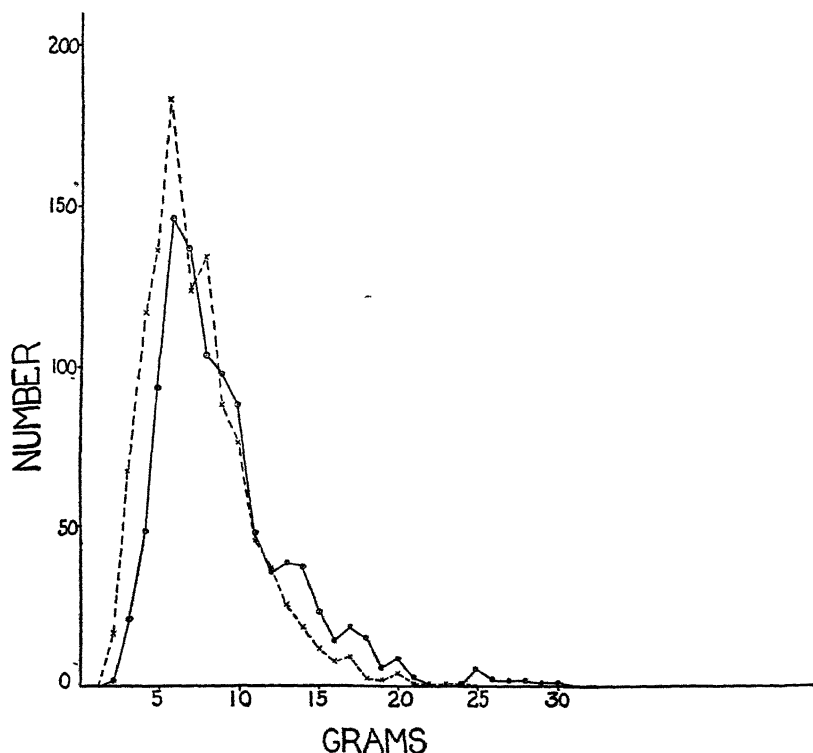


FIGURE 1. Positively skew distributions of mature fruit weights (grams) in two  $F_2$  tomato populations:

| Cross              | Number<br>of<br>plants | Arith-<br>metic<br>means | Geomet-<br>ric<br>means | Median | $g_1 = \frac{k_2}{k_2^{3/2}}$ |
|--------------------|------------------------|--------------------------|-------------------------|--------|-------------------------------|
| 3313 $F_2$ ---x--- | 1107                   | $7.41 \pm .10$           | 6.73                    | 6.24   | $+1.105$                      |
| 3210 $F_2$ —•—     | 994                    | $9.03 \pm .09$           | 8.24                    | 7.49   | $+1.596$                      |

unless interaction takes place, some explanation must be given for the skewness. Obviously RASMUSSEN'S theory does not apply in this case. But if the factors react with one another on a geometric or percentage basis, each adding a definite proportion to the already existing capital, then such addition to a small capital will make very little difference, while the same percentage increases based on a large capital will result in large increases.



The result of such a scheme will be that the weights crowd together at the lower end of the scale and spread out at the upper end. This is just what happens in many of the crosses that we have studied. From the means and statistical constants of the data given in the legend of figure 1 it will be seen that the geometric means of the distributions are much closer to the median than are the arithmetic means. This would indicate that the distribution is almost normal around the geometric mean, so that if the data were plotted logarithmically the curve would be nearly symmetrical.

TABLE 3  
*Mean fruit weights in grams of tall and dwarf  $F_2$  segregates, and their absolute and percentage differences.*

| Tall parent    | Dwarf parent      | Mean $F_2$ fruit weights |           | Differences      |                        |
|----------------|-------------------|--------------------------|-----------|------------------|------------------------|
|                |                   | Tall                     | Dwarf     | Absolute (grams) | Proportional (percent) |
| Red Currant    | ×902 selection    | 6.9±.10                  | 8.4±.30   | 1.4±.31          | 20.5                   |
| Tangerine      | ×902 selection    | 81.8±1.4                 | 101.6±2.0 | 19.8±2.5         | 23.0                   |
| Devon Surprise | ×B. Preserving    | 24.0±1.0                 | 18.1±2.5  | 5.9±2.7          | 26.2                   |
| Yellow Peach   | ×Dwarf Aristocrat | 76.8±1.5                 | 61.2±1.9  | 15.6±2.4         | 21.1                   |
| 740 selection  | ×"Primula"        | 24.8±1.4                 | 18.0±1.3  | 6.8±1.9          | 30.0                   |
| 97M selection  | ×3213 selection   | 54.0±1.4                 | 38.0±2.3  | 16.0±2.7         | 32.0                   |

### 3. THE LINKAGE OF QUALITATIVE AND QUANTITATIVE FACTORS

The results of LINDSTROM (1928) and our own extensive data (mainly unpublished) show that there are a number of definite genetic linkages between the known qualitative factors and fruit size genes; for the present purpose part of the first chromosome linkages are chosen (table 3) to illustrate some novel features of gene relationship. These are all crosses of a dwarf parent with a tall one; in some cases the fruits of the dwarf  $F_2$  segregates are larger than those of the tall ones, while in other cases, where expected, the converse relationship is true. Therefore this linkage of fruit size with the dwarf gene must be interpreted as truly genetic and not physiological. The important point now concerns a comparison of the values in the last two columns of table 3. It will be noted that, whereas the absolute difference in grams (next to the last column) between the means of the two segregate classes varies a great deal, the percent increases (in the last column) are relatively constant. Since this percentage is found by putting the difference between the class means over the  $F_2$  mean it would appear probable that the action of the true fruit size gene or genes linked in this first chromosome is to cause an increase in growth which is proportional to the basic fruit size. This illustrates clearly the

difference in such a situation between simple additive growth and proportional or geometric growth. The geometric type of increase prevails in this case, and our results show further that the same type of relationship is exhibited with at least eleven other genes.

Not only the true size genes, but also those influencing size through shape, or general physiological effects display the same action. In our more recent studies (MACARTHUR 1934 a, 1935) we have observed that at least two factors, fasciated fruit (*f*) and lutescent foliage (*l*), show a physiological association with size. The fasciation factor always tends to make the fruits of the fasciated segregates larger than those of the respective smooth-fruited ones, regardless of whether the fasciated parent is the larger or the smaller fruited. Lutescent, on the other hand, tends to make the fruits smaller than those on the green plants in the same  $F_2$ . It is interesting to note that though the differences of the  $F_2$  class means of the fasciated and smooth in the one case and the lutescent and green in the other are erratic when considered in terms of grams, they are rational and subject to analysis when considered on a percentage basis. The rough fasciated fruits are 60 to 80 percent larger than the regular smooth ones, while the fruits of the lutescent plants are 15 to 20 percent smaller than those of the corresponding green plants, carrying similar residual genes.

It is interesting to anticipate here what is taken up more fully in section four and the discussion, namely, the physiological mechanism involved. The evidence to date regarding the action of the fasciated gene indicates that it exerts its effect in the early primordial stage by proliferating more locules; and indeed it would seem logical to infer from observations of the number of fasciated flowers that contain two, three, or even four separate or partly fused ovaries, that the mechanism is more properly characterized as ovary proliferation rather than locule proliferation. No matter which of these mechanisms is operating the net result of imposing this system on the basic fruit size is the same; that is, the effect of this increase in locule number on final fruit size is proportional to the already determined locule size and final cell size. Lutescent on the other hand seems to retard the later developmental processes so that its effect is also proportional to the capital involved.

#### 4. THE GROWTH OF THE TOMATO FRUIT AS RELATED TO CELL NUMBER AND CELL SIZE

Since 1925 it has been our established practice (without at first realizing its significance) to classify tomato plants segregating for fruit shapes (oval, pear, fasciated, etc.) and in a general way for fruit size by an inspection of the ovary primordium at anthesis. This naturally directed attention to growth phenomena during the early period, and in the summer of 1934

the junior author made a histological study of cell number and cell size during this period and of the influence of these factors on the subsequent development of fruit size. Since the majority of our conclusions, though derived in a different manner, are essentially the same as Miss HOUGHTALING'S (1935) the reader is referred to her paper for a detailed discussion of the influence of cell number and cell size on the mature fruit size.

Our conclusions may be summarized as follows:—

1. The period prior to anthesis is characterized by cell division, and any differences in ovary size at anthesis are associated entirely with cell number and not with cell size.
2. The post-anthesis period is characterized chiefly by cell expansion, cell division being a minor factor which just suffices to maintain the epidermis, the cells of which do not expand.
3. The differential cell expansion takes place early in the post-anthesis period and the maximum cell expansion varies greatly in the several varieties.

Since in arriving at these conclusions we made use of the probable number of cells present at each stage, some of the figures of which are included in table 4, it is advisable to give the method employed in calculating them. The variety Yellow Cherry has an ovary diameter of 1.1 mm and a cell wall thickness of .1 mm, the diameter of the cells at this stage is .0r mm. From these data the following computations are made, assuming the fruit to be a sphere:—

|   |                |        |
|---|----------------|--------|
| External volume $1.1^3 \times .5236$        | = .6969        | cu. mm |
| Internal volume $(1.1 - .2)^3 \times .5236$ | = <u>.3817</u> | cu. mm |
| Volume of ovary wall                        | = .3152        | cu. mm |
| Volume of cell .0r <sup>3</sup>             | = .000001      | cu. mm |
| Number of cells .3152/.000001               | = 315,000      | cells. |

By the above method of calculation the cells in the central septa are omitted but this does not introduce any serious error, and in our estimation is preferable to any method that treats the ovary as a solid body.

Table 4 shows that the tomato species and varieties can be divided into three more or less well defined groups as regards cell numbers, with *L. pimpinellifolium* representing the first group, yellow cherry the second, and the *esculentum* varieties the third. The two F<sub>1</sub> hybrids between Red Currant and *esculentum* types as well as Burbank Preserving, known to be a selection from such a cross, fall into the second or intermediate group. Our more recent data show that all F<sub>1</sub> hybrids of *L. pimpinellifolium* X *L. esculentum* have cell numbers between 300,000 and 400,000 and fall into this class, even when the *esculentum* variety is Beefheart which has a relatively enormous 400 gram fruit.

In general the larger the fruit the greater the number of cells in its ovary; there are however exceptions to this rule which are explained by reference to the fifth column where the diameters of the mature cells are given. Therefore cell number and cell expansion together account for most of the final fruit size, as may be tested by computing the cell mass (from the diameters) and multiplying by the cell number.

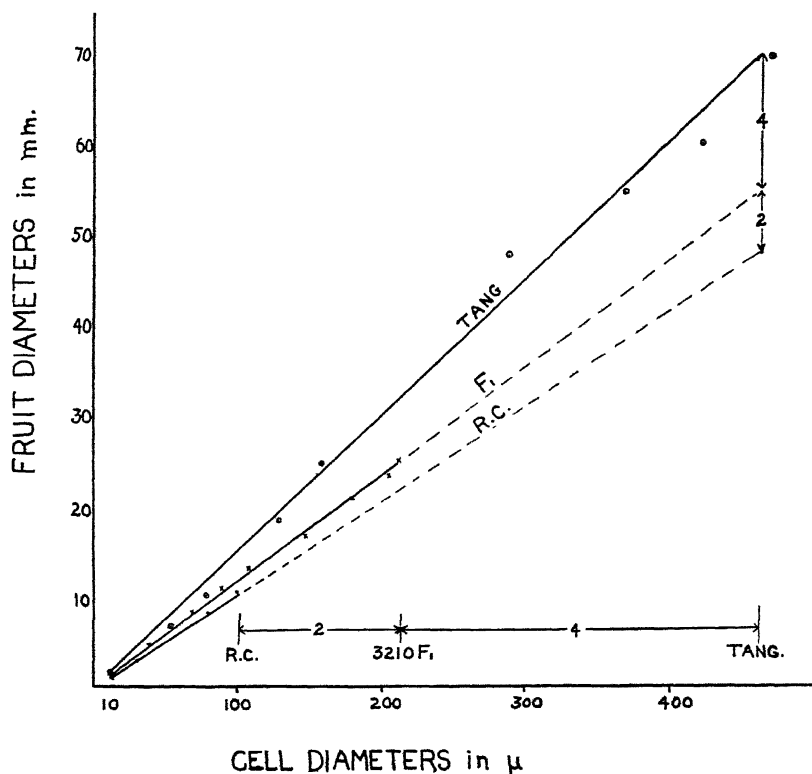


FIGURE 2. Graphs indicating that cell expansion and cell size both operate as geometric processes. A differential increase in cell numbers is seen in the divergent slopes of the three lines and a differential cell expansion in unequal extensions of these lines (see text).

As cell expansion also plays an important part in the determination of fruit size something should be said about its mode of inheritance. Table 4 shows that the mature cell diameters are also divisible into three classes and that these classes bear a geometric relationship to one another.

The effect of these two factors, cell number and cell size, on the final fruit size is brought out clearly in figure 2, where cell diameter is plotted against fruit diameter. The relationship between cell and fruit diameter is evidently a straight line, the slope of the line in the case of the two parents being very different and the  $F_1$  being intermediate. The differences

in slope indicate that for any given cell size the Tangerine has a much larger fruit than does the Red Currant, because of the greater number of cells present in the Tangerine variety. The amount of divergence between the three lines is therefore the effect of initial cell number; the greater the cell expansion the larger the absolute differences between the three lines, but the relative differences remain the same. By observing the end points on each line the effect of cell expansion on fruit size can be studied. If we hold cell number constant, by plotting the final cell sizes all on a line with the same slope, such as the extrapolated Red Currant and  $F_1$  lines,

TABLE 4

*The number of cells in the pericarp wall of tomato fruits at flowering time and the cell diameters in mature fruits. The cell size at anthesis in all varieties is taken as .01 mm.*

| VARIETY                         | FRUIT<br>WEIGHT<br>(GRAMS) | OVARY<br>DIAMETER<br>(MM) | THICKNESS<br>OF WALL<br>(MM) | NUMBER OF<br>CELLS IN<br>THOUSANDS | MATURE CELL<br>DIAMETERS<br>(MICRA) |
|---------------------------------|----------------------------|---------------------------|------------------------------|------------------------------------|-------------------------------------|
| Red Currant                     | 1.1 $\pm$ .005             | .85                       | .086                         | 164                                | 110                                 |
| 3313 $F_1$ (902 $\times$ R.C.)  | 7.2 $\pm$ .06              | 1.08                      | .10                          | 303                                | 200                                 |
| 3210 $F_1$ (Tang $\times$ R.C.) | 8.3 $\pm$ .07              | 1.09                      | .10                          | 309                                | 220                                 |
| Yellow Cherry                   | 4.2 $\pm$ .11              | 1.1                       | .10                          | 315                                | 120                                 |
| Burbank Preserving              | 5.2 $\pm$ .09              | 1.2                       | .09                          | 401                                | 140                                 |
| Yellow Peach                    | 42.6 $\pm$ 2.0             | 1.5                       | .08                          | 507                                | 400                                 |
| Devon Surprise                  | 58.0 $\pm$ 2.2             | 1.4                       | .10                          | 531                                | 400                                 |
| Banalbufor                      | 33.1 $\pm$ 1.0             | 1.5                       | .10                          | 617                                | 350                                 |
| 902 selection                   | 56.2 $\pm$ .05             | 1.6                       | .08                          | 640                                | 400                                 |
| Stirling Castle                 | 98 $\pm$ 5.4               | 1.7                       | .08                          | 659                                | 360                                 |
| Tangerine                       | 173 $\pm$ 3.6              | 1.8                       | .08                          | 745                                | 550                                 |
| John Baer                       | 69.6 $\pm$ 3.4             | 1.8                       | .09                          | 829                                | 470                                 |

we find that the fruit diameters read from these points show geometric progression. Hence we are dealing with two geometric factors, which together would account for the observed relationship between parents and  $F_1$  shown in table 1, and the effect of cell expansion superimposed on cell number is to accentuate the geometric relationship. A similarity may be seen in the time of operation of these two factors, differential cell division taking place in the early part of the pre-anthesis period, and differential cell expansion taking place in the early part (first nine days) of the post-anthesis period.

From the  $P_1$  and  $F_1$  data there at first appeared to be a correlation between cell number and cell size. But in the  $F_2$  population the apparent association between cell number and cell size proved to be a pseudo-correlation; for when the weight is held constant by partial correlation methods

we may safely conclude that cell number and cell expansion are really two separate genetic entities.

Letting  $w$ =mature weight,  $s$ =cell size, and  $n$ =cell number, the correlations are as follows:

$$r_{ws} = +.674 \pm .038$$

$$r_{sn} = +.411 \pm .059$$

$$r_{un} = +.523 \pm .051$$

$$r_{sn.w} = +.0009 \pm .70$$

Thus the study of cell number and size, and of the growth of the tomato fruit through all but the earliest primordial stages of its development, has led to some definite results which may be associated with the genetic findings. Parents of different fruit size, and their reciprocal crosses in  $F_1$ ,  $F_2$  and backcross generations come early to differ in cell numbers in their growth. This early difference in cell number is followed by a subsequent brief period of differential cell expansion. The growth in the long final period is approximately equal both in rate and duration in all varieties and hybrids.

The data presented under headings 1 to 4 draw attention to four lines of approach to the problem of fruit size inheritance, each leading to the same conclusion. Collectively they form a substantial support to the following hypothesis.

#### A WORKING HYPOTHESIS OF FRUIT SIZE INHERITANCE

A view has already been proposed (MACARTHUR 1935, BUTLER 1937) that appears tenable for the tomato data and adequate to explain the principal results described. The basic size factors are considered as rate genes, one group of which governs the rate of division (or the duration of active mitosis) in the formative stages from the earliest establishment of the fruit primordium to anthesis. Earlier or later in this limited time-effective period a large-fruited variety or species undergoes a few more cell divisions than a smaller-fruited one. The differences of cell numbers found in fruits of typical varieties (table 4) are such as would be caused by a difference of one or two divisions out of at the most 17-19 divisions. Each extra division at this stage doubles the initial capital. Ovary proliferation and formation of additional locules may both increase this capital. On the basis of such differences in cell number at anthesis, the size increases occurring later by expansion of each of the cells is therefore proportional to the cell number. Increase of capitals at equal rates over equal periods of time by the compound interest rule results in comparatively vast absolute differences in final fruit sizes. There is, however, the further evidence suggesting that other factors governing cell expansion itself may be also geometric in their action (fig. 2). Since both basic processes, con-

trolling cell number and cell size, are probably geometric in nature, the mature fruit sizes would be such as were observed (tables 1 and 2).

From the genetic aspect, the allelic size factors from parents of different size would control the rate and number of divisions in the corresponding formative period of the  $F_1$  hybrid. Thus a blending of mitotic rates results in the "dominance of small size" (wild type) factors in the fruit of the hybrid, that is, in a geometric mean of ultimate size as far as cell number is involved; a partial dominance of the slower rate would reduce the  $F_1$  size even below the geometric mean (table 1).

A small parent with cells from  $x$  mitotic divisions crossed with a large parent with  $x+2$  divisions would produce an  $F_1$  with fruit size characterized by  $x+1$  divisions. This latter number is also the average of the  $F_2$  generation, but with factor segregation increasing the variability as in the usual theory. To illustrate with an actual case, the cross of the 164,000-celled Red Currant with the 745,000-celled Tangerine was observed to produce an  $F_1$  hybrid bearing fruits of 309,000 cells. In the two backcrosses the expected less-than-intermediate number of cells are evidently formed, as shown by the ovary sizes and the geometric means.

Similar considerations are obviously applicable to cell expansion (data in last column of table 4). In any case a large amount of variation in fruit sizes would be expected for at least two reasons, quite apart from the known modifying effects of environment and of genes controlling shape, locule number, etc (YEAGER 1937): (1) Doubtless mitoses do not continue synchronously in all cells of the anlage, at least after chemo- and histodifferentiation begin. Accordingly cell numbers in varieties and hybrids are not expected to conform strictly to any 1:2:4 series. (2) Cell division and cell expansion appear to be largely independent processes; if so, the possible combinations of cell numbers and sizes would be numerous and varied enough to give a wide range of fruit sizes. An attempt is being made to synthesize and select some of these combinations, for example fruits containing the Red Currant cell number and the Tangerine cell size, etc. Each such selection should theoretically be obtainable with or without fasciation, with high or low locule number, and in varying shapes. Collectively these selections would be expected to duplicate most of the fruit types occurring in the known cultivated varieties and wild species.

#### DISCUSSION

The familiar view of size inheritance in its simplest form assumes that size genes 1) are numerous, 2) lack dominance, producing a blend as regards size in the  $F_1$  hybrid, 3) are equal, and 4) simply additive in their effect, producing symmetrical  $F_2$  distributions, and that 5) their segrega-

tion and assortment explain the increased variance of the  $F_2$  population and the different means and variance of  $F_3$  selections.

Objection is raised only to attributes 2), 3) and 4). The assumed lack of dominance is patently inconsistent with the observed behavior of the bulk of qualitative genes and with the dominance theory of heterosis, which also deals with the same or similar quantitative factors. The cases actually analyzed have often revealed some major and other accessory or modifying size genes. The  $F_2$  distributions are frequently asymmetrical.

In the tomato where the analysis of fruit size has perhaps been carried farther than in other species, genetic studies have from the first shown clearly that the fruit size is gene-transmitted, size as a character being unusually complex and multifactorial. Many typical recessive mutant factors affecting size have been recognized and located in chromosomes (MACARTHUR 1934b); some of these exert their important size effects by modifying fruit shape or locule number or both (YEAGER 1937); for example, the genes for fasciation (*f*) and those raising the locule number increase fruit size, probably by increasing the basic cell number, while at least two genes which elongate the fruit (ovate, *o*, and "plum") tend to decrease its size. Other recessive genes with marked and distinguishing specific qualitative effects also have a general or physiological influence on size (MACARTHUR 1934a, 1935; CASTLE 1936); lutescent (*l*), a chlorophyll deficiency, slows down both plant and fruit growth and decreases the ultimate size of the fruit; tangerine (*t*) and possibly peach (*p*), on the other hand, appear to enlarge the fruit perhaps by acting on the cell expansion mechanism. Linkage experiments have shown the existence of other factors, for example, in chromosomes I, II and III, which presumably act as "size genes" *per se* for they produce their size effects when fruit shape, locule number, and all known qualitative factors are held constant.

These instances show that the several identified factors controlling size obviously do not necessarily or always lack dominance. Many are known to exhibit a fairly typical dominant-recessive relationship. It is relevant to mention also that the dominance theory of hybrid vigor implies an  $F_2$  asymmetry, but not of the kind observed, since the skewness is negative if large size is dominant (ASHBY 1937c, pp. 432-33).

Some of the factors isolated are certainly unequal, for they have been shown to affect size by varying amounts. Several of the genes determining size or number of cells are apparently distinct in nature and mode of influence, inasmuch as they affect different processes and act at different times in ontogeny. The histological analysis of fruit development, though still far from complete, already directs attention not to one process only, but to many (rate and duration and localization of cell division, rate of



advancing differentiation, time and amount of cell expansion, etc). It is certainly clear that some of the chief size factors influence ovary proliferation ( $f$ ), or locule number, others localization of differential growth ( $f$ ,  $o$ , plum); and still others probably determine fruit cell expansion ( $t$ ,  $p$ ) or fruit cell division rate or general plant growth ( $l$ ). These factors being dissimilar in nature and unequal in effect, no linear relation would be expected or possible between the number of factors and the size of the fruits. What they have in common is that most of them act comparatively early in fruit growth, and probably all of them act, whether directly or indirectly, on processes of a geometric nature.

Since the  $F_2$  distributions are strongly and positively skew the interaction of the various size factors can hardly be either arithmetically cumulative in total effect or according to a law of diminishing returns. Both the  $F_1$  positions and the  $F_2$  distributions are features associated with geometric growth, and show the likelihood that consideration of geometric processes will prove indispensable for an understanding of size inheritance. Since cell number and size are of widespread importance in many processes involving embryological organization and physiological functions, it is possible that quantitative characters other than size may also have a geometric basis.

It deserves some notice that conclusions in many ways parallel have been reached recently by several investigators working with quite different materials and objectives. In mutant races of *Drosophila*, an eye size directly proportional to that characteristic of the imago has been fixed and is already detectable in the anlage by the time the first 11.6 percent of the total development period is completed (MEDVEDEV 1935). The breed differences in body size of rabbits are evident when only five or six cleavage divisions have occurred (CASTLE and GREGORY 1929, GREGORY and CASTLE 1931); and in chickens such cell number differences are discernible in the embryos before hatching (BLUNN and GREGORY 1935). The latest work on the physiology of heterosis (ASHBY 1937a,b,c; LUCKWILL 1937; but contrast EAST 1936) has again referred the greater size of  $F_1$  plants displaying a marked hybrid vigor to an initial advantage in size of the embryo primordium. Such cases from both animals and plants suggest that some common and consistent explanation may be found to account for the genetically determined size differences characteristic of parts or of whole organisms.

These observations concentrate attention on the present need for researches 1) to discover just how early and by what factors the differences in amount of capital, that is, the number and size of cells comprising the primordium of the organ anlage or embryo, are determined; 2) to demonstrate more precisely by linkage studies the existence and nature of factors

more or less specifically controlling cell number and cell size, and obtain such factors in their various combinations, and 3) to unify the theory underlying heterosis and quantitative inheritance in general.

#### SUMMARY AND CONCLUSIONS

The tomato provides especially favorable material for the study of fruit size inheritance from the combined genetic and developmental aspects. Many qualitative and quantitative factors have been identified and mapped whose specific or general size effects have been localized as to time, site and general mode of action.

That fruit size fundamentally involves geometric processes is indicated by four different lines of approach:

- 1) The average  $F_1$ ,  $F_2$  and backcross fruit sizes approach more closely to the geometric mean of the parent varieties or species crossed, than to their arithmetic means, as is usually supposed.
- 2) Linked size genes, as well as fruit shape and other genes affecting size, appear to operate on a percentage basis.
- 3) The  $F_2$  distributions are positively skew.
- 4) Histological analysis of developing fruits shows that the basic phenomena involved are those controlling cell number and cell size, both of which appear to act during limited time-effective periods (p. 260), and in a geometrical manner.

The characteristic variety or species fruit sizes are anticipated in the size of the ovary primordium, which in turn is determined by differences in cell number (brought about by different rates of cell division in the pre-anthesis period), and by varying amounts of cell expansion (fixed in the first days after anthesis). On the basis of the unequal cell numbers and sizes established in the early anlage the later observed proportionate or percentage increases produce relatively enormous absolute differences in mature fruit size.

A theory proposed to account for the histological observations and the genetic results is that rate genes control in the main two basic geometric processes, some determining the number of cell divisions, others the amount of cell expansion. Together the factors govern differential growth in size of the primordia, creating unequal amounts of initial capital. It is such genes acting geometrically the heterozygous combinations of which produce the  $F_1$  fruit sizes noted and the assortment of which explains the means and skewness of the  $F_2$  distributions.

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# THE RELATION OF ALBINISM TO BODY SIZE IN MICE

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Received January 24, 1938

**I**N PREVIOUS studies made in coöperation with former colleagues at the BUSSEY INSTITUTION of HARVARD UNIVERSITY, it was found that certain recessive mutant genes, when homozygous, increase the body size of mice, whereas other mutant genes decrease the body size.

In particular the brown (or chocolate) gene was found to increase adult body weight by 3 or 4 percent, and body length by about 1.5 percent. That the brown mutation increases body size was independently discovered and first reported by FELDMAN (1935), who made observations on the relative body weight of black and of brown individuals in three different races of mice in which the two alternative colors were occurring together in the same litter. He gives averages for a group of from 35 to 50 mice of each sex and color, weighed at monthly intervals between the ages of one month and six months. At each of the six weighings in both sexes, the brown mice were heavier than the blacks by from 1.6 to 5.6 percent. Combining the percent differences for both sexes, the series runs thus:

|                                 |     |     |     |     |     |     |
|---------------------------------|-----|-----|-----|-----|-----|-----|
| Age in months.....              | 1   | 2   | 3   | 4   | 5   | 6   |
| Browns heavier, in percent..... | 2.8 | 2.2 | 2.0 | 3.1 | 2.4 | 3.5 |

From this it would seem that the brown gene, in FELDMAN's observations as in our own, when homozygous makes mice heavier by about 3 percent than when it is heterozygous, and further that this influence is of about the same strength at all ages. The growth period studied by FELDMAN covers that from an average weight of about 9 grams at one month of age to a weight of about 23 grams at six months of age in males of race H. In race J the corresponding weights are 10 and 32, and in the third race (IHJ) they are 9.8 and 26.6.

From the fact that the percentage difference is substantially the same at 1, 4, and 6 months of age in FELDMAN's mice, it seems probable that it was already effective at birth or even earlier (as genetic size differences are in rabbits).

The dilution gene also was found by us to increase body weight and body length, though to a less extent than the brown gene, but tail length was in several crosses increased more strongly by dilution than by brown, indicating a special localized action of the dilution gene.

The combined action of the two genes, brown and dilution, was about equal to the sum of their effects when acting separately.

An opposite effect, decrease of body size was found to occur, when the gene for short ear or the gene for pink eye was present in homozygous state. The gene for short ear was thought to reduce body weight by about 5 percent, the gene for pink eye by less than one percent, though the evidence was not altogether clear.

In the case of the agouti gene, no evidence was found that it either increases or decreases body size.

In continuance of this line of research, an experiment has recently been made to ascertain what effect, if any, the albino mutation *c* has on body size. The conclusion reached is that it has no effect, since individuals homozygous for albinism do not differ in average size from their colored litter mates which are heterozygous for albinism. The evidence on which this conclusion is based will now be presented.

In the fall of 1936, in the Veterinary Science Laboratory of the UNIVERSITY OF CALIFORNIA, a cross was made between albino mice of the formula *AA bb cc* and dilute brown colored mice of the formula *aa bb CC*. The albino mice were kindly supplied by Dr. E. C. MACDOWELL, the dilute brown mice were obtained from the Supply Department of the ROSCOE B. JACKSON MEMORIAL LABORATORY. Both races had been long inbred and so would be theoretically of complete genetic uniformity. In making the cross albino females were mated with dilute brown males. The  $F_1$  young, like their albino mothers, were animals of remarkable size, vigor, and fecundity. In color they were cinnamon and their genetic formula obviously would be *Aa bb Cc*. That is, they were heterozygous for agouti and for albinism, but like both parents were homozygous for the brown gene.

$F_1$  females were now backcrossed with males of a triple recessive albino race kindly supplied by Dr. L. C. STRONG, his long inbred race A, which is of the formula *aa bb cc*. The resulting backcross mice fall into four genetic classes expected to be numerically equal one to another, *Aa bb Cc* (cinnamon), *aa bb Cc* (brown), *Aa bb cc* (albinos potentially cinnamon), and *aa bb cc* (albinos potentially brown). The last two classes are indistinguishable in appearance and so there are really only three phenotypes, cinnamon, brown, and albino, and their expected proportions are 1:1:2. In a backcross population of 1252 mice raised to an age of six months, the actual numbers are 334 cinnamon, 308 brown, and 610 albino, a sufficiently close approximation to the expected 1:1:2 ratio.

A comparison of individuals of the three phenotypes should show whether genes *A, a* and *C, c*, in their alternative forms exercise any appreciable influence on body size. If the gene *A* exercises any influence on size different from that of its allele, *a*, then the cinnamon mice (*Aa*) should differ significantly in size from the browns (*aa*). And if gene *C* exercises

on size an influence different from that of its allele,  $c$ , then the average size of the colored mice (cinnamons and browns, which agree in being  $Cc$  in formula) should be different from that of the albinos, which are all  $cc$  in formula.

Animals of the backcross population were weaned at an age of three or four weeks. The sexes were caged separately, about 12 or 15 animals to a cage, and kept constantly supplied with Purina Dog Chow and water. They were weighed individually at monthly intervals from about four months of age, and the maximum weight recorded for each animal was regarded for statistical purposes as its adult weight. In the case of females, which were of course not allowed to breed, the final weight observation made was usually the maximum, or at any rate there was little decline up to six months of age from a maximum previously attained. In the case of males the maximum was often attained as early as four months of age, subsequent to which weight might be lost from fighting but this did not seem to affect either body length or tail length, if the tail remained uninjured. In case the tail was severely injured by fighting, its length was not included in the calculation of average tail length. This accounts in part for the smaller number of animals tabulated as to tail length. But there also occurred a certain number of animals in both sexes which had stubby tails obviously abbreviated at birth by an overzealous mother in the process of cleaning the new born young, or else congenitally shorter and stubbier than normal as to tail form. These also were omitted in tabulating the data on tail length.

The tail length in these backcross mice was measured from the point to which the body fur covers the tail (disregarding the longest contour hairs) to the tail tip (projecting hairs however being here disregarded). The tail measurement was made independently of the body measurement and the difficulty in determining the point on the *morphological* tail to which the body fur extended will account in part (but only in part) for the greater variability of the tail measurement, as compared with that of body length. Actually tail length varies more in relation to body length, than body weight does. This is indicated by the lesser magnitude of the correlation coefficient between tail and body when compared with the correlation coefficient between weight and body. Weighing was done with a Toledo scale, which proved both expeditious and accurate to within 0.2 gram.

When the animals were six months old they were chloroformed and measurements were taken of the body length and tail length of each animal after SUMNER's method, keeping the body slightly stretched under tension of 20 gram weights attached to teeth and tail respectively.

Table 1 contains a summary, for each sex separately, of the observa-

tions on body weight, body length, and tail length. Males are in all three respects larger than females and so are summarized separately, but no phenotype differs significantly from either of the other phenotypes of the same sex in weight, body length, or tail length.

Brown males average a trifle larger bodied by all three criteria than cinnamon males, but the difference is less than twice the probable error

TABLE 1

*Comparative body size in a backcross population, of mice of the three phenotypes, cinnamon, brown and albino, as indicated by body weight, body length and tail length.*

| MALES                 | NO. | AVERAGE<br>WEIGHT | NO. | AVERAGE<br>BODY LENGTH                  | NO. | AVERAGE<br>TAIL LENGTH                 |
|-----------------------|-----|-------------------|-----|---|-----|--|
| Cinnamon              | 173 | 39.45 ± .16       | 172 | 102.66 ± .10                            | 165 | 93.75                                  |
| Brown                 | 153 | 39.91 ± .14       | 153 | 102.92 ± .10                            | 146 | 93.82                                  |
| Cin. and br. combined | 326 | 39.67 ± .09       | 325 | 102.78 ± .07                            | 311 | 93.77                                  |
| Albino                | 310 | 39.54 ± .09       | 310 | 102.58 ± .07                            | 304 | 93.82                                  |
| Total                 | 636 | 39.61 ± .05       | 635 | 102.68 ± .03<br>$\sigma = 1.90 \pm .03$ | 615 | 93.79 ± .07<br>$\sigma = 2.91 \pm .05$ |
| FEMALES               |     |                   |     |   |     |  |
| Cinnamon              | 161 | 30.78             | 161 | 98.38                                   | 161 | 90.89                                  |
| Brown                 | 155 | 31.34             | 155 | 98.10                                   | 154 | 90.68                                  |
| Cin. and br. combined | 316 | 31.09             | 316 | 98.22                                   | 315 | 90.78                                  |
| Albino                | 300 | 31.47             | 300 | 98.75                                   | 295 | 91.02                                  |
| Total                 | 616 | 31.27             | 616 | 98.33                                   | 610 | 90.90                                  |

and so not significant. And in the case of females, this relation is reversed at least as regards body length and tail length, for cinnamon females exceed their brown sisters slightly in these measurements. We may conclude therefore that the *Aa* phenotype does not differ in body size from the *aa* phenotype, which conclusion agrees with that reached in experiments previously reported.

We come now to the prime objective of this experiment, to discover whether albinism has a tendency either to increase or to decrease body size. For this purpose we may compare the average body size of colored individuals with that of their albino litter mates. The combined cinnamon and brown classes constitute the colored individuals, the body size of which is to be compared with that of the albinos. The 326 colored males of table 1 have an average body weight of 39.67 grams; the 310 albino males average 39.54 grams. The difference between these averages is .13 gram, which scarcely exceeds the probable error, .12 gram, and so is not significant.

In body length, the colored males are just .10 mm longer bodied than the albinos, a difference which just equals the probable error. In tail length the difference between colored and albino males is also insignificant, being only .05 gram, actually less than the probable error. Albinism accordingly is without detectable influence on the body size of males.

A similar conclusion is reached in the case of females from a comparison of the body size of colored and of albino females. The average body weight of 316 colored females is 31.09 grams. For 300 albino females, it is .38 gram greater but this is less than the difference in weight between the two colored classes, cinnamon and brown, which on grounds already discussed was not considered significant. Also the relation between the colored and the albino females as regards body weight is the reverse of that observed among the males, since colored males were heavier than albino males, but colored females weigh less than albino females. Probable errors were not calculated for the female population, but if they are substantially the same as for the corresponding groups of males, the difference in weight between colored and albino groups would not have statistical significance.

The albino females, as regards body length and tail length, as well as body weight, are slightly larger bodied than their colored sisters, but this relation is doubtless a consequence of random sampling and not indicative of genetic differences, as is shown by the following considerations. As the data were accumulated, they were from time to time summarized. Three such partial summaries were made, about 200 individuals being included in each. In two of these summaries the average weight of the brown females was greater than that of the cinnamons, but in the third summary the cinnamons were heavier than the browns. Also in two of the partial summaries colored females are heavier than albinos, but in the third summary albinos are heavier than colored individuals.

That it is through general rather than local growth processes that genes commonly influence body size is indicated by the positive correlations which exist between body weight, body length, and tail length. An individual which is large by one of these criteria is also large by the other criteria, and an individual which is small by one criterion is also small by the others. This is true even within inbred races and populations derived by crossing such inbred races, as in the present experiment. Here genetic uniformity is nearly complete and such variability as exists must be referred almost wholly to accidents of development. Organic correlations are regularly less within inbred populations where genetic influences are uniform than in other populations in which genetic influences are variable. For example, in the backcross population described by CASTLE, GATES and REED (1936), where several genes affecting body size were segregating, the correlation between body weight and body length was found to be



$.65 \pm .01$  in the case of females, and  $.66 \pm .01$  in the case of males. But in the present experiment in which variation is uninfluenced by genes affecting body size, the corresponding correlation for males is only  $.55 \pm .01$ .

Tail length shows a greater degree of independent variability than either weight or body length. The correlation between body length and tail length was found to be only  $.26 \pm .02$  in the available male population of 613 individuals, in which were included only those with uninjured tails. In a previous publication the gene mutation dilution was found to exert a direct influence on tail length, in addition to the indirect influence which in common with the brown mutation it exerts through its action on general growth. In the present experiment homozygous dilution does not occur in the backcross population, so this complication is avoided.

#### SUMMARY

An experimental test was made of the influence of the albino mutation on the body size of mice. Albino females of an inbred race (*AA<sup>b</sup>bcc*) were crossed with dilute brown males (*aabbCC*). The  $F_1$  mice, cinnamon in color, were *AabbCc*.  $F_1$  females were backcrossed to triple recessive inbred males *aabbcc*. Mice of three phenotypes were produced, cinnamon (*AabbCc*), brown (*aabbCc*), and albinos (either *Aabbcc* or *aabbcc*). These three phenotypes in a backcross population of 1252 animals occurred in the expected ratio, 1:1:2. The animals were grown under uniform conditions to an age of six months, then killed and measured as to body length and tail length, having been previously weighed at monthly intervals. The body size of each individual was judged by three criteria, maximum weight at or prior to six months of age, body length and tail length.

No significant difference was found between body size, as estimated by any one of these criteria, among the three phenotypes. In particular the colored classes were neither larger nor smaller bodied than the albinos. The conclusion is reached that albinism (and incidentally also the non-agouti mutation) is without influence on body size. Among the 635 male individuals the correlation between weight and body length was found to be  $.55 \pm .01$ . Among 613 available males the correlation between body length and tail length was found to be  $.26 \pm .02$ .

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# NEW MUTANTS IN SCIARA AND THEIR GENETIC BEHAVIOR

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Received January 31, 1938

THE present paper deals with six new mutant characters in *Sciara* found in this laboratory. In addition, two mutants are recorded which appear to be identical with or allelic to two of these characters. Of the six, one is an autosomal dominant in *Sciara coprophila* Lintner, and one a sex-linked recessive in *Sciara ocellaris* Comstock. The remaining four, including one autosomal dominant, two sex-linked dominants, and one sex-linked recessive, are in *Sciara reynoldsi* Metz (METZ, in press). Selective segregation of chromosomes has previously been established in three species of *Sciara* (METZ 1926, 1928, 1929). The present paper establishes selective segregation in two other species; namely, *S. ocellaris* and *S. reynoldsi*.

The dominant characters were found by the second author among the descendants of flies which had been exposed to radium. The radiation was done at the Howard A. Kelly Hospital in Baltimore through the kindness of Dr. FRED WEST, to whom we are greatly indebted. Whether the mutations were produced as a result of the radiation cannot be stated definitely,

TABLE I  
*Linkage data for the character "Stop" in S. coprophila.*

| TEST          | CASES | TOTAL<br>FLIES | CLASSIFICATION OF FLIES |                  |                   |   | PERCENTAGE OF<br>CROSSING OVER |
|---------------|-------|----------------|-------------------------|------------------|-------------------|---|--------------------------------|
| $D \times F$  | 16    | 1177           | <i>D</i><br>297         | <i>F</i><br>279  | <i>DF</i><br>301  | + | Not linked                     |
| $S \times C$  | 9     | 405            | <i>S</i><br>109         | <i>C</i><br>73   | <i>SC</i><br>80   | + | Not linked                     |
| $S \times F$  | 18    | 688            | <i>S</i><br>338         | <i>F</i><br>349  | <i>SF</i><br>1    | + | 0.15                           |
| $S \times Dl$ | 32    | 1160           | <i>S</i><br>587         | <i>Dl</i><br>570 | <i>SDl</i><br>1   | + | 0.26                           |
| $S \times B$  | 22    | 852            | <i>S</i><br>445         | <i>B</i><br>404  | <i>SB</i><br>0    | + | 0.35                           |
| $S \times D$  | 12    | 393            | <i>S</i><br>152         | <i>D</i><br>190  | <i>SD</i><br>21   | + | 12.98                          |
| $S \times tr$ | 24    | 1357           | <i>S</i><br>563         | <i>tr</i><br>414 | <i>Str</i><br>183 | + | 28.00                          |

since *Sciara* has proved to be very resistant to radiation. Since the characters are dominants, it seems probable that some of them, at least, were produced at the time of radiation. Otherwise, they should have appeared previously in the stock cultures.

The sex-linked recessive in *S. reynoldsi* was found by the first author, and the genetical tests, analyses, and descriptions presented here (with the exception of the sex-linked recessive in *S. ocellaris*, which was found and analyzed by Mrs. E. GAY LAWRENCE) are by the first author.

### I. The mutant character *Stop* in *S. coprophila*

a) *Origin*. *Stop* is an autosomal dominant which was found on February 20, 1936, in a female, the mother of which had been treated with 8 gm. hrs. of radium.

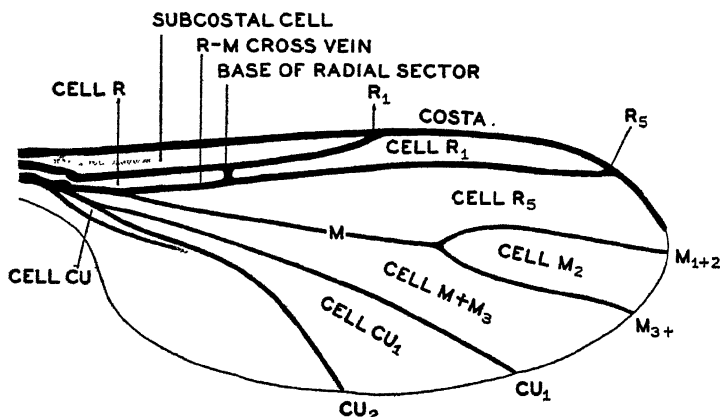


FIGURE 1. Diagram of normal *Sciara* wing. Terminology after Johannsen.  $R_1$ , first branch of radius;  $R_5$ , posterior branch of radius;  $M$ , media;  $M_{1+2}$ , anterior branch of media;  $M_{3+}$ , posterior branch of media;  $CU_1$ , anterior branch of cubitus;  $CU_2$ , posterior branch of cubitus.

b) *Description*. In the *Stop* wing (see fig. 1 for diagram of normal *Sciara* wing) the posterior branch of the radius vein and the anterior and posterior branches of the media vein end before they reach the wing margin. *Stop* is a clear-cut and constant character, which can be recognized easily in crosses that involve other wing characters. *Stop* flies exhibit excellent viability as shown by the fact that heterozygous *Stop* females crossed to wild type males give progenies which closely approximate a 1:1 ratio. Seven pair matings of this type selected at random gave 132 wild type to 118 *Stop* flies.

c) *Genetic behavior of males*. The transmission of *Stop* through males follows the typical *Sciara* pattern, that is, males transmit only the genes derived from their mothers. From crosses of wild type virgin females to

Stop males which received Stop from their mothers, the offspring are all Stop. On the other hand, the offspring from crosses of wild type virgin females to Stop males which received Stop from their fathers, are all wild type.

d) *Linkage*. Genetic tests, corroborated by cytological observations, as shown below, indicate that Stop involves a translocation between chromosomes II and IV.

Crosses of virgin wild type females to Stop males (which received Stop from their mother) revealed that Stop was not sex-linked. If it were sex-linked, the  $F_1$  males from such a cross would all be wild type, since the paternal X is eliminated from the soma cells in the male at about the eighth cleavage division (DuBois 1933). The  $F_1$  males from such a cross, however, are all Stop, indicating that the Stop character is an autosomal dominant. Three autosomal linkage groups have been identified in *S. coprophila* (SMITH-STOCKING 1936): chromosome II, with the characters *truncate* and *Dash*; chromosome III, with the *Curly* character; and chromosome IV, with *Blister*, *Delta*, and *Fused*.

(1) *Tests with Chromosome III*: Stop  $\times$  Curly ( $S \times C$ ). Tests summarized in table 1 show that Stop is not in Chromosome III.

(2) *Tests with Chromosome IV*: Stop  $\times$  Fused ( $S \times F$ ). The data, given in table 1, indicate that Stop is "in" chromosome IV and closely linked to Fused.

Stop  $\times$  Delta ( $S \times D\Delta$ ), and Stop  $\times$  Blister ( $S \times B$ ). Similarly, Stop was found to be linked to Delta and Blister. From table 1 we find the crossover value between Stop and Delta to be 0.26 percent, while that between Stop and Blister is 0.35 percent. Delta and Blister are probably alleles. SMITH-STOCKING (1936) reports 6.9 percent crossing over between Delta and Fused and 7.0 percent crossing over between Blister and Fused.

(3) *Tests with Chromosome II*: Stop  $\times$  Dash ( $S \times D$ ). Crosses between Stop and Dash revealed that Stop is linked also to a character in chromosome II. Tests between Stop and Dash gave a crossover value of 12.98 percent between Stop and Dash. The question arose as to whether Dash was really in a linkage group distinct from Blister, Delta, and Fused. SMITH-STOCKING's report (1936) gives clear-cut evidence that neither Blister nor Delta is linked to Dash; however, no direct tests between Fused and Dash are recorded. In order to clarify this point, tests were made between Dash and Fused. Classifying the offspring from these crosses was somewhat complicated since Fused obliterates one of the characteristic effects of the Dash gene, that is, the "Dash" vein situated between the anterior and posterior branches of the cubitus vein. However, Fused does not obliterate the brownish marking along the posterior branch of the radius which is also a characteristic effect of Dash. The tests be-

tween Dash and Fused indicate definitely that Dash and Fused are located in different chromosomes.

Stop  $\times$  truncate ( $S \times tr$ ). Tests between Stop and truncate gave further proof that Stop is linked to characters in chromosome II as well as to those in chromosome IV. Such tests gave a crossover value of 28 percent between Stop and truncate.

e) *Conclusion*. In view of the genetic evidence presented above, Stop is believed to be due to (or accompanied by) a translocation between chromosomes II and IV. Cytological examination of the salivary gland chromosomes of heterozygous Stop flies shows that there is a translocation between two of the four pairs of synapsed somatic chromosomes. This translocation makes it possible to identify cytologically the four linkage groups in *S. coprophila*; the translocation distinguishes chromosomes II and IV, and the X chromosome is distinguishable from chromosome III by its unpaired condition in the male soma (that is, in salivary gland cells).

The Stop translocation apparently affects crossing over in both chromosomes, II and IV. In chromosome IV SMITH-STOCKING (1936) found 6.9 percent crossing over between Delta and Fused and 7.0 percent crossing over between Blister and Fused. From table 1 we find the crossover value between Stop and Fused to be 0.15 percent, between Stop and Delta 0.26 percent, and between Stop and Blister 0.35 percent. It appears, therefore, that the Stop translocation cuts down crossing over in chromosome IV. On the other hand, Stop apparently increases crossing over in chromosome II, since the crossover value between Stop and Dash is 12.98 percent and that between Stop and truncate 28 percent; while the crossover value between truncate and Dash reported by SMITH-STOCKING (1936) is only 2.8 percent.

## II. The mutant character "yellow" in *S. ocellaris*

a) *Origin*. A yellow male was found in a normal wild type culture of *Sciara ocellaris* on February 11, 1935 by Mrs. E. GAY LAWRENCE.

b) *Description*. Yellow is a body color which extends to most parts of the fly except the eyes and bristles. It is clear-cut and constant and can be readily distinguished from wild type.

c) *Genetic behavior*. Yellow behaves as a sex-linked recessive (first analyzed by Mrs. E. G. LAWRENCE). Yellow females from the monogenic strain give either male or female families (a few "exceptional" males and females are found). Thus, if a yellow female-producing female is mated to wild type males, all of her daughters should be phenotypically wild type. On the other hand, if a yellow male-producing female is mated to wild type males, all of her sons should be yellow, since the paternal sex chromosome is normally eliminated from the male soma at an early cleav-

age stage (DuBois 1933). However, it may be mentioned here that there are many cases of irregular sex-chromosome elimination in this yellow strain (unpublished data of Mrs. E. GAY LAWRENCE and of CROUSE). For instance, a number of males have been found which retained the paternal sex chromosome in their soma instead of the maternal sex chromosome.<sup>1</sup> Normally *Sciara* males transmit only those characters which are maternal in origin. An attempt has been made to determine whether these irregular males which retain the paternal sex chromosome in the soma line transmit this chromosome, but all such males tested have proved to be sterile.

### III. Mutant characters in *S. reynoldsi*

#### 1. Puff

a) *Origin.* Puff is an autosomal dominant which appeared in a digenic strain of *S. reynoldsi*. It was found on January 4, 1936 in a male, the mother of which had been treated with 5 grm. hrs. of radium.

b) *Description and genetic behavior.* Puff is very similar to the character "Blister" in *S. coprophila*. It appears as a blister or vesicle at the juncture of the posterior and anterior branches of the media vein. The Puff character is variable; it ranges all the way from a large blister to a slight swelling, and in some cases overlaps normal. Fifteen pair matings of Puff females  $\times$  wild type males, selected at random, gave the following offspring: 294 wild type females, 204 Puff females, 283 wild type males, 165 Puff males, that is, approximately 63 offspring per female. On the other hand, fifteen pair matings selected at random of wild type females by wild type males gave 515 females and 903 males in the  $F_1$ , that is, approximately 95 offspring per female.

Homozygous Puff females have never been found. Puff females derived from the cross, Puff female  $\times$  Puff male (Puff from mother), have all proved to be heterozygous. Apparently, therefore, Puff is lethal in homozygous condition. The transmission of Puff through the male line agrees with that of other characters in *Sciara*. Puff males which receive the character from their mother transmit it to all their offspring, whereas Puff males which receive Puff from their father do not transmit it at all.

#### 2. Vesiculated

a) *Origin.* A Vesiculated female was found on January 6, 1936 in a digenic culture of *S. reynoldsi*. The parents of this Vesiculated fly had been exposed to 3 grm. hrs. of radium.

<sup>1</sup> Such irregular elimination does not appear to be associated with the yellow locus itself, since the paternal sex chromosome retained in some cases has carried yellow and in other cases its normal allele.

b) *Description and genetic behavior.* In appearance, Vesiculated is identical with Puff; it is likewise variable, ranging all the way from a large blister to a normal juncture of the posterior and anterior branches of the media vein. Like Puff, Vesiculated is lethal in homozygous condition. However, it is much less viable when heterozygous. From ten pair matings selected at random of Vesiculated females  $\times$  wild type males, there were 123 wild type females, 72 Vesiculated females, 64 wild type males, and 35 Vesiculated males in the  $F_1$ , that is, approximately 29 offspring per female. To determine whether Puff and Vesiculated are alleles is practically impossible, since the two characters are so similar. However, Puff females were mated to Vesiculated males (the reciprocal cross was made also) and all of the  $F_1$  females backcrossed to wild type. Without exception, each mating gave two types of offspring: wild type and Puff (or Vesiculated?). That is, in no case were Puff and Vesiculated obtained in the same fly. This fact, together with the fact that neither Puff nor Vesiculated has been found in homozygous condition, suggests that the two characters are allelic or perhaps identical. The considerable difference in viability makes it probable that they are not identical.

### 3. Jagged and Jagged-2

a) *Origin.* A Jagged female appeared in a digenic culture of *S. reynoldsi* on January 6, 1936. Its parents had been treated with 5 grm. hrs. of radium.

b) *Description.* The Jagged character appears as nicks or incisions along the wing margin between the posterior branch of the cubitus vein and the anterior branch of the media vein. The position and number (usually one or two) of these marginal nicks are somewhat variable, and in some instances the Jagged character is detectable only by missing marginal hairs. Jagged showed poor viability from the time of its origin, and the stock was finally lost.

c) *Genetic behavior.* The first Jagged female found was mated to wild type males. The offspring from this cross fell into three groups: wild type females, Jagged females, and wild type males. During the eighteen months that Jagged was kept in the laboratory, the offspring from Jagged females mated to wild type males always fell into these three groups. No Jagged males ever appeared. This fact indicates that Jagged is a sex-linked dominant which is lethal in homozygous condition, and also lethal in the male where one sex chromosome is present in the soma.

d) *Jagged-2.* On the same day that Jagged appeared, Jagged-2 was found in an  $F_1$  female of the same digenic stock of *S. reynoldsi*. The parents of this J-2 fly had been treated with 5.4 grm. hrs. of radium. The J-2

character proved to be identical with Jagged in appearance, viability, and genetic behavior.

#### 4. Ruffled

a) *Origin*. A Ruffled male was found in January, 1936, in a digenic strain of *S. reynoldsi*. The parents of this male had been treated with 2.9 grm. hrs. of radium.

b) *Description*. In the female heterozygote the wings appear stiff and are characterized by a wave approximately midway of their length. The degree of waviness varies, and in many cases the female heterozygotes are not distinguishable from wild type. On the other hand, the female homozygotes never overlap normal. The wings of these flies are wavy, extremely wrinkled, and have something of the appearance of the wings of newly-hatched imagos. In other words, the wing size, shape, and venation are altered in the female homozygotes. The wing of the Ruffled male seems to occupy a position intermediate between these heterozygotic and homozygotic extremes. The wings are only slightly wrinkled, and the venation can be seen. However, the wave in the wing is distinct, and Ruffled males can always be distinguished from wild type.

c) *Genetic behavior*. Ruffled shows the same type of inheritance as other sex-linked dominants in *Sciara*.

#### 5. Yellow

a) *Origin*. A yellow Ruffled male was found in a mass culture of Ruffled stock on October 23, 1936 by the first author.

b) *Description*. Yellow in *reynoldsi*, like yellow in *ocellaris*, is a body color which extends to most parts of the fly except the eyes and bristles.

c) *Genetic behavior*. The original yellow Ruffled male was mated to wild type females. The  $F_1$  generation consisted of 60 Ruffled females and 33 wild type males, showing that yellow is a recessive. These Ruffled  $F_1$  females backcrossed to wild type males gave, without exception, four classes of offspring: wild type males, yellow Ruffled males, wild type females, and Ruffled females. Yellow thus behaved as a sex-linked recessive. When these  $F_2$  Ruffled females (carrying yellow) were mated to yellow Ruffled males, yellow Ruffled females, Ruffled females, yellow Ruffled males, and wild type males were obtained. During the twelve months that yellow has been kept in the laboratory, it has always appeared with Ruffled. Evidently little, if any, crossing over occurs between the two loci. Due to the decreased viability of homozygous Ruffled, the stock carrying yellow has to be kept outcrossed. Apparently the yellow gene does not decrease viability, since yellow Ruffled flies are as viable as Ruffled flies.



The fact that *S. ocellaris* and *S. reynoldsi* can be hybridized (METZ and LAWRENCE in press) has made it possible to cross *ocellaris* yellow and *reynoldsi* yellow and to demonstrate that the two genes are alleles.

#### ACKNOWLEDGMENT

The authors are indebted to Dr. C. W. METZ for suggestive criticism and to Mrs. ELIZABETH GAY LAWRENCE for making some of the genetic tests.

#### SUMMARY

Six new mutants have been found in *Sciara*, among descendants of radium-treated flies. Stop, an autosomal dominant in *S. coprophila*, involves a translocation between chromosomes II and IV. Yellow is a sex-linked recessive in *S. ocellaris*. Four mutations have been found in *S. reynoldsi*: Puff and Vesiculated, autosomal dominants which are lethal when homozygous and may be allelic or perhaps identical; Jagged, a sex-linked dominant, lethal when homozygous; Ruffled, a sex-linked dominant; and yellow, a sex-linked recessive closely linked to Ruffled.

The yellow mutations of *S. reynoldsi* and *S. ocellaris* are alleles.

The genetic behavior of these characters establishes selective segregation in *S. ocellaris* and *S. reynoldsi*.

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# SALIVARY CHROMOSOME STUDIES OF LETHALS IN *DROSOPHILA MELANOGASTER*

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Received February 2, 1938

IT IS very difficult to define a lethal except in the wide genetical sense of being a factor which brings about the death of the organism. Supposedly every genetic lethal has its physiological or morphological counterpart which prevents the organism from living beyond a certain stage, but little is known about these lethals themselves—whether they are genic or chromosomal changes, are closely allied to mutations, are additions to or losses from the non-lethal constitution. The evidence in *Drosophila* indicates that the types of changes which are genetically detectable form a graded series; cases are known from one extreme, where the change results in decreased viability, to the other extreme, where the change is both lethal to the organism as a whole and to the individual cell, and all intermediate types have been encountered. Thus lethals can be classified according to the degree of their effect. Even more complex is any attempt to correlate genetic lethals with cytological changes since cases are known of lethals appearing in salivary gland chromosomes as deficiencies (SLIZYNSKA 1938), of genetically tested lethals having no effect on the known banding (SLIZYNSKA 1938), and of visible cytological deficiencies not having any lethal effect at all (DEMEREK and HOOVER 1936). It is clear then that the study of lethals is from many points of view an interesting one. The approach to these problems chosen here is that of parallel genetical and cytological studies which are possible in *Drosophila melanogaster*. This organism is particularly suitable for such a study because the frequency of occurrence of both spontaneous and induced lethals is relatively high.

## PROBLEM AND MATERIAL

In the study of lethals interest is focused upon three primary problems: (1) what lethals are, (2) how they occur, (3) how, when, and where they act. The third problem comprises a large field of embryological and physiological investigations, but the first and second problems can be approached partially from the cytogenetic standpoint. Light may perhaps be shed on these questions by two lines of study. (a) Comparison of spontaneous and induced lethals, and (b) study of the relationship of genetic lethals to cytological deficiencies. The present paper attempts to give the results obtained from a particular study along these lines.

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Nineteen lethals which had arisen spontaneously were studied. These lethals had been collected over a period of many years by Dr. M. DEMEREC and were maintained in his stocks carried over Inversion dl-49. Thirteen lethals induced by X-ray treatment in experiments by the same investigator and similarly maintained in stocks were taken for comparison. All the lethals used were sex-linked and located to the left of garnet.

#### METHODS

Since previous work has shown that some of the induced lethals are cytologically detectable, the two investigations mentioned above may be attacked simultaneously by studying the salivary gland chromosomes of mature larvae known by genetic tests to carry lethals which have arisen spontaneously. In a majority of cases the lethal was subjected to the standard crossover tests in order to determine its approximate genetic position:

(1)  $l/dl-49, \gamma Hw m^2 g^4$  by  $ec ct^6 v g^2$ .

(2)  $F_1 l/ec ct^6 v g^2$  by  $dl-49, \gamma Hw m^2 g^4$  and counts were made of all females and males. In other cases as will be discussed later special crossover tests were necessary.

After the location within certain limits was thus known, each lethal was studied cytologically in the salivary gland chromosomes. Preparations were made from mature female larvae. For this purpose the stock females  $l/dl-49, \gamma Hw m^2 g^4$  were outcrossed to  $g^2 B \underline{XY''} \underline{YY''}$  males. In the  $F_1$ , two types of females are recovered, one being homozygous and the other heterozygous for  $g$ . The former in the larval stage show white Malpighian tubules and the latter have tubules yellow in pigmentation. By utilizing this difference it was possible to select always the larvae carrying the lethal. Slides were prepared according to the aceto-carmin method and were made permanent by the alcohol-euparal technique described by BAUER (1936). For studying the material use was made of a Zeiss 90 $\times$ , 1.4 N.A. apochromatic objective, and oil immersed achromatic 1.4 N.A. condenser, compensating oculars (10 and 12.5 $\times$ ), a Bausch and Lomb research lamp (ANTHES 1936; BRIDGES 1936) equipped with a Wratten filter No. 62.

Both synapsed diploid and unsynapsed haploid chromosomes were studied, in every case using only good and well-stretched figures. Each determination was of necessity checked by other persons in the laboratory, including C. B. BRIDGES, M. DEMEREC and B. P. KAUFMANN. The fact that in some cases deficiencies were not detected does not constitute definite proof that deficiencies do not exist in that particular material. Negative evidence on such a point is not conclusive. Furthermore, it should be mentioned here that no attempt was made in the scope of this work to

make exact determinations as to the limits of each deficiency. It was considered sufficient for the present purpose to determine the occurrence of a deficiency without pursuing a more specialized study.

## RESULTS

In table 1 is given a summary of the genetical and cytological data for the 32 lethals studied. They are listed in order of location from left to right of the chromosome. Cytological deficiencies are noted in all cases where they were detected; each analysis was verified as noted by several investigators. The reference system is to BRIDGES' (1935) salivary gland chromosome map except in some instances when Dr. BRIDGES furnished a revision of certain regions. The genetic location, as already mentioned, was determined by crossing over tests for which the data are presented in detail. Several special crosses were made:

291-44  $l/rb\ cx \times dl-49, y\ Hw\ m^2\ g^4$

$\sigma^7\sigma^7$ : non-crossovers—288;  $cx$ —4;  $rb$ —24

This shows that the lethal lies between  $rb$  and  $cx$  in the ratio 24:4 corresponding to the locus 8.4.

291-3  $l/rb\ cx \times dl-49, y\ Hw\ m^2\ g^4$

$\sigma^7\sigma^7$ : non-crossovers—485;  $cx$ —30; +—6

This shows that the lethal lies to the right of and close to  $cx$  at approximately 14.8.

291-7  $l/t^2\ v\ f \times dl-49, y\ Hw\ m^2\ g^4$

$\sigma^7\sigma^7$ : non-crossovers—207;  $v\ f$ —2;  $t^2$ —7;  $t^2\ v$ —51

This shows that the lethal lies between  $t$  and  $v$  in the ratio 2:7 corresponding to the locus 28.7.

291-9  $l/ct\ v\ dy\ g\ f \times dl-49, y\ Hw\ m^2\ g^4$

$\sigma^7\sigma^7$ : non-crossovers—936;  $ct\ v\ dy$ —62;  $v\ dy$ —2

This shows a slight reduction in crossing over between  $dy$  and  $g$  and considerable reduction between  $ct$  and  $v$ . No chromosomal aberration is present but it is possible that in addition to the deficiency in 8C, bands may be missing in 7D.

291-11  $l/ct\ v\ dy\ g \times dl-49, y\ Hw\ m^2\ g^4$

$\sigma^7\sigma^7$ : non-crossovers—705;  $v\ dy\ g$ —88;  $dy\ g$ —33;  
 $g$ —4;  $ct\ v\ dy$ —64;  $v\ dy$ —2

This shows that the lethal lies between  $dy$  and  $g$  in the ratio 66:4 corresponding to the locus 36.7. Salivary gland chromosome analysis here led to the suspicion of a very minute inversion and this possibility is not excluded.

291-53  $l/ct\ v\ dy\ g\ f \times dl-49, y\ Hw\ m^2\ g^4$

$\sigma^7\sigma^7$ : non-crossovers—199;  $v\ dy\ g\ f$ —23;  $dy\ g\ f$ —20;  $g\ f$ —5;  
 $f$ —3;  $ct\ v$ —2;  $ct\ v\ dy$ —4;  $ct\ v\ dy\ g$ —28; *wings-folded*—9.

TABLE I  
Summary of genetical and cytological data for spontaneous and induced lethals.

| NUMBER | DEFICIENCY<br>FOR | LOCATION    | ec<br>v | ec<br>g | ec<br>v | ec<br>g | ec<br>v | ec<br>g | ec<br>v | ec<br>g | cl<br>v | cl<br>g | cl<br>v | cl<br>g | ec<br>v | ec<br>g | cl<br>v | cl<br>g | lethal lies<br>between<br>in ratio |
|--------|-------------------|-------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|------------------------------------|
| 291-54 | —                 | Spontaneous | 33      | 4       | 7       | 7       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | 62: 7; see text                    |
| -58    | 4A4*              | left of ec  | 364     | 61      | 4       | 4       | 7       | 7       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -44    | —                 | 7.0         | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -100   | —                 | 8.4         | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -3     | 5C4.5*            | ec-cl       | 40      | 6       | 9       | 13      | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -95    | 5D3.4†            | ec-cl       | 85      | 6       | 12      | 6       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -82    | —                 | ec-cl       | 77      | 5       | 2       | 6       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| 296-14 | —                 | 18.8        | 50      | 1       | 14      | 9       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -15    | —                 | 21.0        | 108     | —       | 12      | 15      | 19      | 1       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -8     | —                 | 22.0        | 95      | —       | 16      | 17      | 12      | 2       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -13    | —                 | 23.9        | 109     | —       | 11      | 22      | 26      | 6       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -19    | —                 | 24.3        | 67      | —       | 4       | 5       | 13      | 2       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| 291-69 | 7E9.10†           | 25.2        | 83      | —       | 12      | 6       | 20      | 8       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -7     | in8B†             | 27.2        | 68      | —       | 3       | 27      | 23      | 18      | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -9     | in8C*             | cl-v        | 24      | —       | 4       | 13      | 15      | 17      | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -11    | in12E*            | 37.0        | 66      | —       | —       | 0       | 14      | 15      | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -53    | in13B*            | 40.8        | 73      | —       | —       | 8       | 7       | 5       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| 296-22 | —                 | 41.4        | 165     | —       | —       | 7       | 20      | 27      | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| 291-5  | 14B6*             | right of g  | 69      | —       | —       | —       | 13      | 13      | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| 294-44 | —                 | Induced     | 69      | 9       | 15      | 7       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| 302-1  | —                 | ec          | 37      | 11      | 9       | 9       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -9     | —                 | ec          | 75      | 13      | 8       | 7       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| 304-69 | 4A1.2†            | 7.0         | 28      | 11      | 5       | 5       | 2       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| 302-3  | in4C*             | 7.7         | 156     | 14      | 7       | 9       | 7       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| 294-22 | —                 | 10.3        | 156     | 16      | 12      | 3       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -27    | —                 | 15.2        | 108     | 6       | 17      | 23      | 12      | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| 304-71 | —                 | 25.0        | 80      | —       | 5       | 13      | 10      | 3       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| 294-16 | 10B8*             | 26.0        | 186     | —       | 16      | 15      | 44      | 18      | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -31    | in10C*            | 33.5        | 111     | —       | —       | 21      | 25      | 12      | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| 304-68 | —                 | 33.7        | 55      | —       | —       | 15      | 19      | 22      | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
| -60    | —                 | 34.1        | 51      | —       | 4       | 10      | 19      | 16      | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |
|        | —                 | 43.0        | 67      | —       | —       | 1       | 16      | 20      | —       | —       | —       | —       | —       | —       | —       | —       | —       | —       | —                                  |

\* Analysis verified by M. Demerec and B. P. Kaufmann.

† Analysis verified by C. B. Bridges, M. Demerec and B. P. Kaufmann.

This shows that the lethal lies between *dy* and *g* in the ratio 4:5 corresponding to the locus 40.8. The "lethal" males which also carry a cytologically detectable deficiency will come through under excellent food conditions. These males have folded wings and are sterile and very weak.

$$291.5 \text{ } l/g^2 \text{ } un^3 f \times dl-49, y \text{ } Hw \text{ } m^2 \text{ } g^4$$

$$\sigma^7 \text{ } \sigma^7: \text{non-crossovers—199; } g^2\text{—27}$$

This shows that the lethal lies to the right of and very close to *g*.

The cytological evidence is summarized in the chart shown in figure 1. In this chart the location of induced and spontaneous lethals is indicated both on the linkage map and on the salivary chromosome map and drawings of critical salivary chromosome figures are shown. In summarizing the cytological data of 19 spontaneous lethals, 9 or 47.3 percent are detectable deficiencies. Among the 13 induced lethals 4 or 30 percent are deficiencies. This value agrees fairly well with the 40 percent obtained by SAKHAROV (1935) and ALIKHANIAN (1937).

#### DISCUSSION

It would seem impossible to escape from the fact already mentioned that in any consideration of lethals a whole range of types of changes presents itself. The present work also provides evidence that genetic lethals may or may not be detectable cytologically. Whether this is because such minute deficiencies are undetectable with available facilities is not certain. Possibly some changes in genes may so affect their physiological activity as to produce a genetic lethal effect, whereas the activity of that gene as expressed in its ability to be represented by nucleic acid production in the form of a salivary chromosome band remains unaffected. In other words, the various lines of activity of a gene are differently sensitive to the environment. Cases are already known of cytological deficiencies having no genetic effect (DEMEREK and HOOVER 1936), just the reverse of the above condition. The hypothesis may be offered that all mutations, deficiencies, and all other types of change are merely expressions of the differential response of the various lines of activity of the genes and that accordingly all these types could be classified and arranged in a more or less orderly series. Be that as it may, evidence is available here that many of the genetically detectable lethals are cytological deficiencies. It is significant that this is true of both spontaneous and induced lethals, and that in this respect the two types of lethals do not differ. This stands in contradiction to the suggestion sometimes made that induced lethals are separable in type from the spontaneous ones. So far as it is known there is no difference between the spontaneous and induced lethals which have been studied in known loci. For example, in the Notch region where a total of 12 lethals were studied by SLIZYNSKA (1938) the

two spontaneous ones presented no evident distinction as to size of deficiency or its effect. Some of the gene changes produced by X-ray treat-

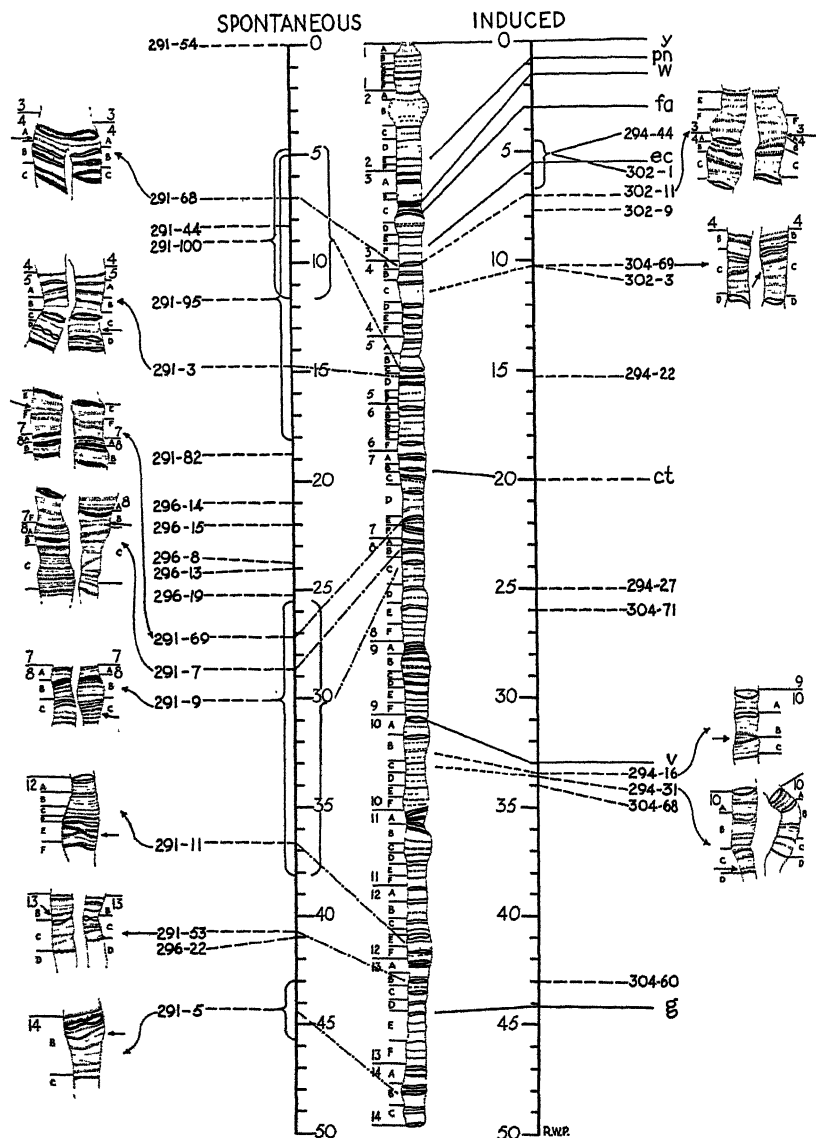


FIGURE 1. Chart indicating location of studied lethals on the linkage map and when known on the salivary chromosome map. Critical figures indicating deficiencies are shown for certain lethals.

ment are in every respect identical with those obtained spontaneously. Similarly here the indications are that induced and spontaneous lethals are alike.

DEMEREK (1937) has suggested that when the frequency of deficiencies is low they would not be expected to be connected with chromosomal aberrations, and that the proportion of aberrations would necessarily increase with an increase in the frequency of deficiencies. Hence the failure of available data to indicate an association between chromosomal aberrations and spontaneous lethals is explicable because the frequency of spontaneous lethals is too low. If this is true, another difference noted between spontaneous and induced lethals is removed.

The results of the present work are not in agreement with those of SAKHAROV (1935, 1936), who studied 27 lethals induced by X-ray treatment and 25 spontaneous lethals all sex-linked. Among the induced lethals, SAKHAROV found 17 chromosomal aberrations. Among the other 10 he found 4 instances of deficiencies. Among the spontaneous lethals he found no instances at all of aberration or deficiency. SAKHAROV and NAUMENKO (1936) also reported studies of lethals produced by treatment with iodine and manganese and again no cases of cytological disturbance were found. The conclusion is consequently drawn that spontaneous and chemical lethals are of a different type than those induced by X-ray treatment. Since in the results recorded here a minimum of 16 percent of spontaneous lethals and deficiencies was found, the conclusions above do not fall into line.

It may be noted with reference to the chart in figure 1 that lines drawn to connect the approximate loci of the lethals or known loci on the cytological map with similar loci on the genetic or crossing over map are not always parallel.

#### SUMMARY

Nineteen cases of spontaneous lethal changes in the X chromosome of *Drosophila melanogaster* were studied. Their genetic locations were determined by standard crossover tests and their cytology studied in salivary gland chromosomes. Of these, 47.3 percent were found to be minute deficiencies. Among the 13 induced sex-linked lethals studied for comparison 30 percent were detectable deficiencies. These results would seem to indicate that there is no primary distinction between spontaneous and induced lethals.

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# SALIVARY CHROMOSOME ANALYSIS OF THE WHITE-FACET REGION OF *DROSOPHILA MELANOGASTER*

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Received February 2, 1938

THE white-facet region of the X chromosome has been utilized extensively in both genetic and cytological studies. It is interesting genetically because of the unique series of alleles recognizable in the white locus and because of the series of alleles and the dominance of the deficiency for the facet locus. It is interesting cytologically also because of the grouping of heavy bands visible in salivary gland chromosomes. In this paper, the results of genetical and cytological studies of fourteen deficiencies in that region are reported.

Except for N-8 (MOHR 1923), 264-36, 264-37, 264-38, and 264-39, the material for all other deficiencies and the data for a number of genetic tests were obtained from M. DEMEREC. N-8 (MOHR) and 264-38 originated spontaneously while all other deficiencies were induced by X-ray treatment. Critical salivary chromosome figures were checked by C. B. BRIDGES, M. DEMEREC and B. P. KAUFMANN. Photographs and redrawing of figures were done by Miss R. W. PARKER.

## METHODS

In order to obtain Notch deficiencies, normal males carrying yellow ( $y=0.0$ ) as a marker were irradiated with approximately 2500 r-units, and then were mated with females homozygous for cherry ( $w^{ch}=1.5$ ) and wavy ( $wy=40.7$ ). From  $F_1$  females the white or Notch flies were selected for this work.

H. BAUER'S (1936) method for preparing salivary glands was followed. Full grown larvae were dissected in aceto-carmine; needles and forceps of stainless steel were used to avoid contamination by iron. Slides were made permanent by the alcohol-euparal technique. Microscopical observations were made using a 2 mm. 1.4 N.A. apochromatic objective, an oil immersed achromatic 1.4 condenser, and 12.5 $\times$  or 15 $\times$  compensating oculars. A Bausch and Lomb research lamp equipped with a green Wratten filter No. 62 was used as a source of light. Drawings were made with the aid of a camera lucida.

Non-synapsed chromosomes were selected for study, because even good preparations of the region in question usually show such distortion that identification of bands is very difficult. In non-synapsed chromosomes the number of bands involved was determined and the length of the deleted

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part was found by comparing the deficiency with a normal haploid strand. In many cases the bands of this region were identified easily by their characteristic size and shape, as well as by the intensity of their staining. To reduce any possibility of error, however, the limits of the deficiency were determined also in the synapsed condition of the chromosome. In that case, the exact position of some deficiencies was determined by combining them with the 258-18 translocation, in which the tip of X chromosome to 3C4 inclusive is transferred to the spindle fibre region of the fourth chromosome, exchanging places with the remainder of the fourth (DEMEREK and SLIZYNSKA 1937). This gave a clearer picture of synapsis in the one end of the deficiency and at the same time it usually prevented synapsis at the other end. But all these methods of examination sometimes failed, especially when the studied band was very faint and lay in a very narrow space between two heavy darkly stained bands or vesicles.

#### RESULTS

The results of both breeding and cytological studies are summarized in figure 1. For a detailed description, BRIDGES' salivary gland chromosome map and his reference system (BRIDGES, 1938) were used. According to this system, every vesicle is counted and numbered as two separate bands.

258-11. This deficiency, as shown by breeding results, includes only one known locus, white, and extends in its cytological picture from 3A3 to 3C2.3 inclusive. In this case it is impossible to determine whether the 3C4 band is present or not, because this band is so faint that it is rarely visible even in the normal chromosomes. It was seen only twice during this work. 258-11, therefore, involves fifteen or sixteen bands.

258-14. Genetically identical to the former, this deficiency is a little shorter cytologically. The first band involved in the deficiency is 3A4, and the last band deficient without any doubt is 3C1. However, the possibility that 3C2 is also absent has not been excluded.

N-8 (*Mohr*). Cytological data in regard to this deficiency differ in some respects from those which are given by MACKENSEN (1935) and GOTTSCHESKI (1937). According to MACKENSEN, this deficiency does not involve 3C1, but involves the double band 3E1 and 3E2. On the contrary, GOTTSCHESKI shifts the limits to the left and places the deficiency from 3C1 to 3D4 inclusive. According to the data presented here, the deficiency extends from 3C1.2 to 3D5.6 inclusive. This determination is based on studies of both synapsed and non-synapsed strands. In haploid chromosomes with N-8 deficiency, between section 3B and 3E, only one dark band is present which may be 3C1 as well as 3E1.2. Although the general features of the band in question seemed to indicate the second possibility, additional preparations were made with the translocation 258-

264-38. This is the largest deficiency described in this paper; it involves 45 bands, from 2D4 to 3E1·2 inclusive, and according to breeding results involves the loci *pn*, *w*, *rst*, *fa*, and *dm*. The females heterozygous for this deficiency show greatly reduced viability as compared with all other Notch cases, and even in good culture conditions they are distinctly smaller than other females. The drawing and photomicrograph figures 2k and 2l show the 264-38 deficiency as it appears in diploid condition, with the small loop formed by the normal haploid.

264-36. This deficiency involves the loci *w*, *rst*, *fa*, and *dm*. Cytologically it covers the section from 3A4 to 3D2 inclusive, containing 25 bands. A drawing and a photograph of that deficiency in a heterozygous condition is shown in figures 2a and 2b.

264-30. The photomicrograph in figure 2j shows a haploid strand of this deficiency. As is indicated on the corresponding drawing, figure 2i immediately following the group of dark bands 3A1 to 3A4 is a much lighter dotted line 3C9·10, not clearly visible on the photograph. The band 3C8 is probably present but since it was seen only in oblique light, this determination was marked as questionable on the diagram in figure 1. The deficiency includes 17 or 18 bands.

264-31. This deficiency, genetically, removes the loci *w* to *dm* inclusive. Cytologically, fourteen bands from 3C1 to 3D2 inclusive are missing.

264-32. This deficiency involves only the *rst* and *fa* loci. The left end starts between 3C2·3 and 3C5·6. The limit in regard to 3C4 is not determined exactly for the same reason as in the case of 258-11. The last band included in this deficiency of 3 or 4 bands is 3C7.

264-37, -39, -33, -2, -19. These five deficiencies are identical with one another in every respect. All were tested with *fa*, *fa*<sup>n</sup>, *spl*, and *Ax* and showed the deficiency for them but not for other adjacent loci. Cytological observation disclosed in all these cases a short deficiency for only one band, 3C7. On photomicrograph figure 2f and drawing figure 2e, 264-39 is represented. The bands 3C1 to 3C5·6 appear as three dark, wide bands. The next band, 3C7, present in the left haploid does not have a corresponding band on the right side. The band 3C8 not well seen on the photograph is again present in both strands and the next two bands (3C9·10), which are clearly visible, are the last synapsed bands on this figure.

264-8. In this case, although breeding tests indicate that *fa*, *fa*<sup>n</sup>, *spl*, and *Ax* loci are deficient, the cytological analysis did not disclose any visible deficiency. In homozygous condition, it produces a lethal effect not only for the whole organism but, according to the data obtained by DEMEREC (1934), it shows a cell-lethal effect in the hypodermal cells of females. The females heterozygous for the 264-8 deficiency show the whole complex of changes characteristic for other Notches. Since the region studied in this case consists of very dark bands usually fused together, the examination of single haploids will show best whether all bands are present. In order to obtain a higher number of non-synapsed strands and to identify the deficient chromosome, N-females of the genetical constitution *N/dl-49*, *y Hw m<sup>2</sup> g<sup>4</sup>* were mated with males carrying the 258-18 translocation. Of three chromosomes involved in this cross, one contains the *dl-49* inversion, another the translocation X-4 so that the identification of the third chromosome carrying the change in the N-region does not

present any difficulty. The photomicrograph figure 2h and drawing in figure 2g, made from the preparation of female larvae from the cross described above, represent the section of a haploid strand of the N-chromosome. It shows clearly that all bands known for this region are present. Moreover, the distance between the single bands and the general appearance of the bands as to size, width, and intensity of staining, do not differ from those which were observed for the normal X chromosome.

#### DISCUSSION

The cytological and breeding results are summarized on the chart of figure 1. On this chart a close relationship between genetical and cytological pictures of the deficiencies studied is evident, indicating that the deficiencies which are similar in their genetical features always have at least one band in common. This serves as a means for determining the position of various loci in salivary gland chromosomes. In this study the problem is limited to the region of the X chromosome from *pn* to *ec*. This section of the chromosome has also been studied by MACKENSEN (1935), GRÜNEBERG (1937), EMMENS (1937), and GOTTSCHESKI (1937). A comparison of the cytological results of different authors, however, presents difficulties because different reference systems are used in different papers.

The limits of the section in which prune (*pn*) is located are given by the left end limits of the 264-38 and 258-11 deficiencies. According to that evidence, the gene is located somewhere between 2D3 and 3A3. MACKENSEN's determination is closer because he designates for this gene the region 2D6 to 2F2 inclusive which probably corresponds on the revised BRIDGES' map to 2D3 or 2D4 to 2F2.

For the location of white (*w*-1.5) evidence was obtained from 258-14, *N*-8, and 264-31 deficiencies. *N*-8 and 264-31 place this locus to the right of the 3B4 band, because 3C1 is the first missing band in both these cases. The 258-14 deficiency, which also includes the *w* locus, shows that this gene must be located to the left of 3C3 since this band lies beyond the limits of the deficiency. A number of figures examined during these studies indicate that 258-14 is not deficient for the 3C2 band, and if this is true, the band 3C1 represents the white locus.

According to GRÜNEBERG (1937) roughest (*rst*) is located 0.2 to the right of *w*. The same author states that *rst*<sup>3</sup> is associated with a long inversion. EMMENS (1937) determined both limits of this inversion, the left one being between 3C2.3 (designated by him 3C2) and 3C5.6 (designated 3C3) and the right in the inert region to the right of *bb*. EMMENS also studied *rst*<sup>2</sup> and described it as a deficiency for bands 3C4 to 3C7 inclusive. The deficiencies described here throw some light on this question. The deficiency 258-11, which does not include *rst*, shows that *rst* should

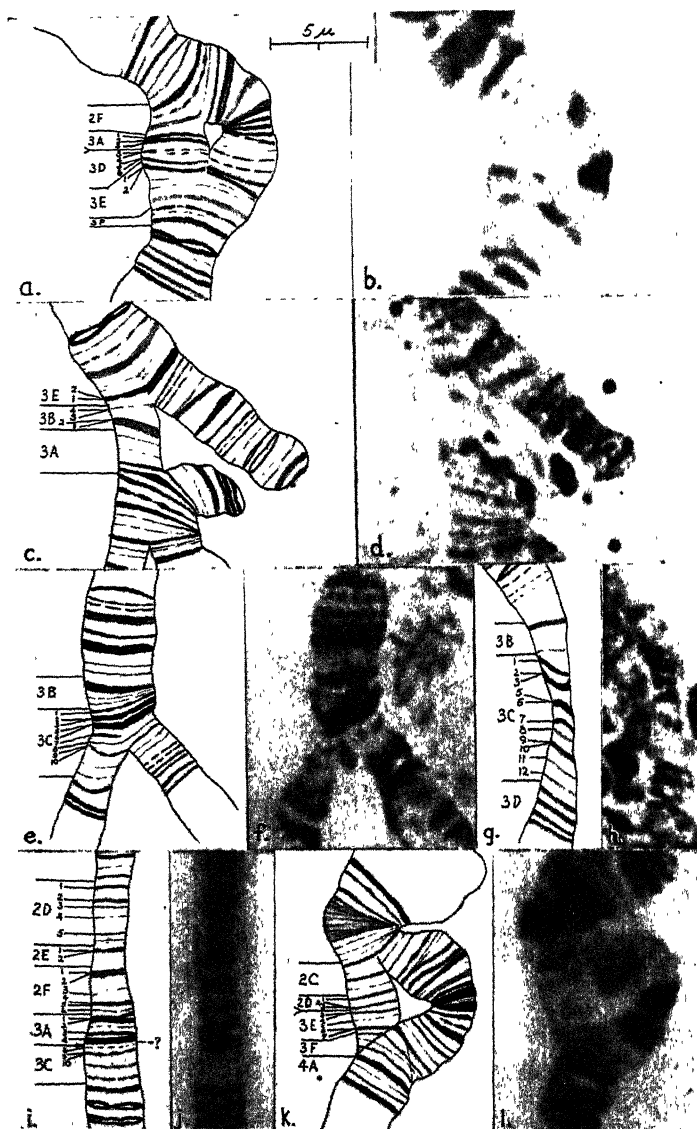


FIGURE 2. Drawings and photographs of various deficiencies. (a) and (b) 264-36 deficiency involving *w*, *rst*, *fa* and *dm* loci and cytologically a section of 25 bands, from 3D<sub>4</sub> to 3D<sub>2</sub> inclusive; (c) and (d) N-8 (Mohr) heterozygous with X-4 translocation 258-18 involving 18 bands, 3C<sub>1</sub>·2 to 3D<sub>5</sub>·6; (e) and (f) 264-38, one band deficiency; (g) and (h) haploid strand of 264-8 showing all bands; (i) and (j) 264-30 deficiency involving 17 or 18 bands, 3A<sub>5</sub> to 3C<sub>7</sub> or 3C<sub>8</sub>; (k) and (l) 264-38, the longest deficiency of this series involving 45 bands, 2D<sub>4</sub> to 2E<sub>1</sub>·2.

be to the right of its right end, namely to the right of 3C2·3. The deficiency 264-32, which includes *rst* and *N* according to the genetical data, starts following 3C2·3 and includes 3C5·6 and 3C7, indicating that *rst* is located between 3C2·3 and 3C8. Deficiencies 264-33, -37, -39, -2, and -19 all of which include the 3C7 band but do not include *rst* show that *rst* is located to the left of 3C7, namely that it is represented either by band 3C4 or 3C5·6. Cytological analysis of translocation 258-18 (DEMEREK and SLIZYNSKA, 1937) shows that the break in the X chromosome occurred genetically after *rst* and cytologically between 3C2·3 and 3C5·6. Since 3C2·3 cannot represent *rst*, as shown by the deficiency 258-11, it seems probable that *rst* is represented by 3C4.

Facet, facet-notched, split, and Abruptex loci will be discussed together because their behavior in crosses with Notch is identical. It is known that notches are deficiencies for all these loci. The results of MACKENSEN place the locus for these genes at bands 3C4 to 3C7 or even probably 3C8. EMMENS locates it at 3C9·10, and GOTTSCHESKI put the genes *co*, *fa*, *spl*, and *Ax* in bands 3C5, 3C6, 3C7, and 3C8 respectively. The material used for our study includes five cases of notch deficiency, each including only the genes mentioned above. Cytologically they all show a deficiency for only one band, 3C7. If it is kept in mind that all cytologically known deficiencies occupying that band are, genetically, deficiencies for all these loci, it can be assumed that all these are represented by this one line. Therefore, there are two probabilities supported by cytological evidence: either these genes are allelic to each other, or one band covers several loci. Genetical data, however, are in favor of the first possibility.

The locus of diminutive (*dm*) should be placed to the left of the right end of 264-31 which includes it.

MACKENSEN (1935) has placed the position of the echinus (*ec*) locus in the region between 3E2 and 3E4. None of the deficiencies mentioned here includes *ec*, therefore, this locus must lie to the right of the right end of the longest of them, which is the 264-38; its righthand limit follows 3E2.

Notch 264-8 presents an interesting case of a genetical deficiency without any detectable change in the salivary gland chromosome. A similar Notch deficiency was first described by MACKENSEN (1935). To explain this phenomenon he assumed a dominant mutation with a Notch character. A second case studied in more detail was the Notch-G case described by GOTTSCHESKI (1937), who states that cytological examination showed a normal condition in the notch region. To explain the case, GOTTSCHESKI discusses three possibilities. First is the chain mutation hypothesis wherein gene changes have occurred in all the involved loci, producing allelomorphs, which, when crossed with respective recessive genes, give the illusion of pseudodominancy. Since this possibility requires several in-



volved assumptions he does not think it is probable. As a second possible explanation for removing the genes without the destruction of normal banding he suggests that some other agent might be responsible for the bands, which also seems improbable. He assumes, as the best explanation, the working hypothesis of inactivation, wherein the gene, although physiologically inactive, does not prohibit the formation of the band. For the present, no better explanation of the problem discussed here seems available.

Among the deficiencies studied, two are spontaneous and twelve induced. One of the spontaneous, 264-39, involves a single band; the other, N-8 Mohr, a block of 18 bands. Five induced deficiencies involve only one band while the remaining six cover larger sections. Although the number of cases studied is small, it appears that spontaneous deficiencies are similar to induced.

If the crossing over map for the region studied is compared with the approximate location of the genes in salivary chromosomes, an interesting fact may be observed. The distances between genes on the standard map do not correspond with those in salivary chromosomes. For example, the distance between  $y$  (0.0) and  $w$  (1.5) is the same on the crossing over map as that between  $w$  and  $fa$  (3.0). In the salivary gland, however,  $y$  is located at the tip of the X chromosome (M. DEMEREC and M. E. HOOVER, 1936) and  $w$  within the bands 3C1 or 3C2, so that the section limited by them is at least 12 times longer than the section between  $w$  and  $fa$  (3C7). It is difficult to determine what is responsible for this discrepancy. Between  $y$  and  $w$  there are about 75 bands, and between  $w$  and  $fa$  there are only about 5. The relation, therefore, is 15:1. The number of bands between  $y$  and  $pn$  is 57, if counted to the middle of the region ascribed for this locus, and the crossing over distance between  $y$  and  $pn$  is 0.8; between  $pn$  and  $w$  there are 18 bands and 0.7 crossing over units; between  $w$  and  $rst$  there are two bands and 0.2 crossing over units; between  $rst$  and  $fa$  there are two bands and 1.3 crossing over units. If we now calculate how many crossing over units correspond to one band in each segment separately we will obtain for  $y$ - $pn$ , the value 0.014; for  $pn$ - $w$ , 0.038; for  $w$ - $rst$ , 0.1; and for  $rst$ - $fa$ , 0.65. It is striking that the numbers are constantly increasing toward  $fa$ .

#### SUMMARY

Salivary chromosome studies of fourteen deficiencies affecting  $w$  and  $fa$  loci were made. The results are summarized in figure 1.

An approximate determination of the position on the salivary chromosome map for the loci involved was made. The locus  $pn$  (0.8) is placed between 3D4 and 3A2;  $w$  (1.5) is represented by 3C1 or 3C2;  $rst$  (1.7) by

3C4; *fa*, *spl*, and *Ax* (3.0) by 3C7; *dm* (4.5) is placed between 3C9 and 3D2 inclusive; and *ec* (5.5) to the right of 3E2.

One case of genetical deficiency without any visible effect in salivary glands chromosome structure is described.

The relations between the crossing over map and the salivary chromosome structure are discussed.

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# THE INDUCTION OF OÖGONIAL CROSSING OVER IN *DROSOPHILA MELANOGASTER*<sup>1</sup>

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Received February 4, 1938

## INTRODUCTION

OÖGONIAL crossing over may be defined as homologous exchange between chromosomes in oögonial cells. Its existence has been suspected because of the similarities between somatic crossing over, induced increases in crossover values in females, the recently demonstrated spermatogonial crossing over in males (FRIESEN 1936, STERN and DOAN 1936, WHITTINGHILL 1937) and also because of the simultaneous appearances of rare crossovers in untreated females (SEREBROVSKY 1927). The similarities are that recombinations are produced or increased by the same agents, high temperatures and X-rays, and that the recombinations are found clustered in the descendent body tissues or offspring.

Recognition of oögonial crossing over by means of like recombination offspring is difficult because the individual cells of such groups are variously altered by the numerous, subsequent meiotic exchanges and are thereby distributed among many recombination classes. If these regular meiotic exchanges could be suppressed in females, as they normally are in males of *Drosophila*, without using such chromosomal irregularities as inversions and translocations, oögonial crossing over might thereby be unmasked. Such an agent is available in the recessive asynaptic factor, c3G, found by GOWEN and located in the third chromosome group.

The action of c3G on crossing over in the different chromosomes is of special interest. GOWEN (1933) found a very low frequency of crossing over: about 1 per 1000 between *st* and *ca* in chromosome III and between *al* and *sp* in II, and about 1 in 1800 for the first three regions of the series *sc ec ct v g f* in the sex chromosomes. These crossovers were for regions away from the spindle attachment in all cases except half of those in chromosome III. Although meiotic crossing over is almost entirely inhibited, somatic crossing over, revealing itself in the "mutant" spots recorded earlier by GOWEN (1929), seems to be unaffected. Hence with respect to crossing over c3G females are comparable to normal males of *Drosophila melanogaster*. If oögonial crossing over could be induced in the former, it would be as easily detectable as spermatogonial crossing over has been in the latter.

<sup>1</sup> Experiment carried out while holding a National Research Council Fellowship in Biology at the California Institute of Technology.

The writer is indebted to the National Research Council for the opportunities of a year's fellowship, during which this work was performed, and to members of the Kerckhoff Biological Laboratories of the CALIFORNIA INSTITUTE OF TECHNOLOGY for suggestions and criticism of methods and of interpretations.

## EXPERIMENTAL PROCEDURE

It was planned to backcross, after treatment with X-rays, females heterozygous for alternated "X<sup>g</sup>" but homozygous for c<sub>3</sub>G to the multiple recessive "X<sup>g</sup>" males; but in the course of making up the stocks not all of the mutants *sc cv v f bb* and *ec ct<sup>b</sup> s car* were retained in all the stocks used. However, all F<sub>1</sub> females were marked with *v f/car* as a minimum.

After irradiation by 2500 r the F<sub>1</sub> females were mated individually in vial cultures to several "X<sup>g</sup>" males. All were changed to four successive cultures, and more males often added, at the end of 6, 10, 14 and 18 days from the time of X-raying, whether fertile or not. Cultures were kept at 25°C and were examined finally on the 17th day. All crossovers were verified by mating again to "X<sup>g</sup>."

## RESULTS

Among 10,826 offspring 16 recombinations grouped in only 11 of the 133 families were found. The crossovers and total offspring of these 11 treated females are given in table 1 by cultures. One female, g, proved to have been a triploid, the only one found in the F<sub>1</sub>. Two females, a and d, were definitely XXY, as shown by the viability of one or more patroclinous males, which carried *bb<sup>1</sup>* in the X. For the same reason at least 11 other females which did not produce any crossovers must have been XXY. The remainder, 9 and 111 from the crossover and non-crossover-producing groups, respectively, did not definitely show the presence of an extra Y, for although matroclinous offspring were numerous in many families, they were more probably primary exceptions due to the action of c<sub>3</sub>G on disjunction.

An index of the gonial origin of a majority of the crossovers was found (table 1) in the pronounced grouping of the recombination classes. Of all the recombinations for the *ec-ct* interval, three were found in one family, the other in a second. For the *cv-ct* interval in more fully marked females, two of the crossovers, which were reciprocals, were found in one family, the other elsewhere. In the next region, *ct-v*, the six crossovers were distributed among only five progenies. In the *s-f* region, as in that between *cv* and *ct*, only 3 crossovers were found among the progeny of 133 females, yet 2 of them occurred in one family. Clearly these clustered crossovers cannot be accounted for by independent occurrences of crossing over in

TABLE 1  
*Crossover-producing families.*

| Successive cultures→<br>Days after x-raying→       |  | 1st<br>0-6                              | 2nd<br>6-10    | 3rd<br>10-14    | 4th<br>14-18   | 5th<br>18-23   | TOTALS           |
|--|--|---|----------------|-----------------|----------------|----------------|------------------|
| COMPOSITION OF<br>F <sub>1</sub> C3G FEMALES       | RECOMBINATION<br>CLASSES                     | TOTAL RECOMBINATIONS<br>TOTAL OFFSPRING |                |                 |                |                |                  |
| a. $\frac{vf}{ec\ ct^s\ car}$ Y                    | $ct^s\ car$                                  | 25                                      | 48             | $\frac{2}{60}$  | $\frac{1}{57}$ | —              | $\frac{3}{190}$  |
| b. $\frac{vf}{ec\ ct^s\ car}$                      | $ct^s\ car$                                  | 13                                      | $\frac{1}{20}$ | 30              | 27             | 24             | $\frac{1}{120}$  |
| c. $\frac{sc\ cv\ vf}{ec\ ct^s\ s\ car}$           | $sc\ cv\ ct^s\ s\ car$<br>$ec\ vf\ Haplo-IV$ | —                                       | —              | 49              | $\frac{1}{42}$ | $\frac{1}{45}$ | $\frac{1}{136}$  |
| d. $\frac{sc\ cv\ vf}{ec\ ct^s\ s\ car}$ Y         | $sc\ cv\ ct^s\ s\ car$                       | $\frac{1}{55}$                          | —              | 29              | 26             | 9              | $\frac{1}{119}$  |
| e. $\frac{vf}{ec\ ct^s\ car}$                      | $car$  | —                                       | —              | $\frac{1}{36}$  | $\frac{1}{26}$ | 3              | $\frac{2}{65}$   |
| f. $\frac{sc\ cv\ vf}{ct^s\ s\ car}$               | $sc\ cv\ s\ car$                             | —                                       | 19             | 19              | 2              | $\frac{1}{3}$  | $\frac{1}{43}$   |
| g. $\frac{sc\ cv\ vf}{ec\ ct^s\ car}$              | $sc\ cv\ car$                                | —                                       | $\frac{1}{50}$ | 14              | —              | —              | $\frac{1}{64}$   |
| h. $\frac{vf}{ct^s\ s\ car}$                       | $s\ car$                                     | —                                       | —              | 30              | $\frac{1}{50}$ | 39             | $\frac{1}{119}$  |
| i. $\frac{vf}{ec\ ct^s\ car}$                      | $car$  | —                                       | —              | —               | $\frac{1}{30}$ | 14             | $\frac{1}{44}$   |
| j. $\frac{vf}{ct^s\ s\ car}$                       | $ct^s\ s\ f$                                 | 57                                      | 66             | $\frac{1}{43}$  | $\frac{1}{68}$ | 3              | $\frac{2}{237}$  |
| k. $\frac{vf}{ct^s\ s\ car}$<br>(in mass cultures) | $ct^s\ s\ f$                                 | 252                                     | 317            | $\frac{1}{387}$ | 319            | 216            | $\frac{1}{1491}$ |

meiosis. Rather each cluster of recombinations (table 2) should be attributed to a single occurrence of gonial crossing over in a single female.

TABLE 2  
*Crossovers and crossing over by region and family.*

| REGION                           | FAMILIES IN WHICH CROSSEOVERS OCCURRED |        |        |       | TOTAL<br>CROSSEOVERS |
|----------------------------------|--|--------|--------|-------|----------------------|
|                                  | SINGLY                                 | DOUBLY | TRIPLY | TOTAL |                      |
| $ec-ct$                          | 1                                      |        | 1      | 2     | 4                    |
| $cv-ct$                          | 1                                      | 1      |        | 2     | 3                    |
| $ct-s$                           | 4                                      | 1      |        | 5     | 6                    |
| $s-f$                            | 1                                      | 1      |        | 2     | 3                    |
| Totals                           | 7                                      | 3      | 1      | 11    | 16                   |
| Non-crossover families and flies |  |        |        | 122   | 10,810               |

TABLE 3  
*Crossovers in relation to time of X-raying.*

| CULTURES<br>Days after X-raying | 1st<br>0-6 | 2nd<br>6-10 | 3rd<br>10-14 | 4th<br>14-18 | 5th<br>18-23 | TOTALS |
|---------------------------------|------------|-------------|--------------|--------------|--------------|--------|
| Oögonial crossovers             | —          | —           | 4            | 4            | 1            | 9      |
| Possibly meiotics               | 1          | 2           | 1            | 2            | 1            | 7      |

The gonial origin of these multiple occurring crossovers was further indicated by the time of their appearance (table 3). If all induced crossing over occurred during the time of exposure to X-rays, the recombinations produced in the younger cells would appear in the later hatches of offspring. It is significant that the clustered crossovers did not appear in the earlier cultures but did appear in those started ten or more days after X-raying. Although there were but few crossovers, they apparently took longer to appear than those induced by PLOUGH (1917) and by MAJOR and SVENSON (1924) in females and as long as the more pronounced spermatogonial crossovers found by FRIESEN (1936). On PLOUGH's calculations, all cells which were oöcytes when X-rayed would have been laid before the tenth day, which is the earliest that one of these oögonial crossovers could have been deposited as an egg.

A third check on the stage of development at which our crossovers were produced was the distribution of mutations. Some occurred singly; others were found bunched and always in the third and fourth cultures. Scute alleles, with normal salivary chromosomes, were found in two families, in one of which it had been transmitted to eight offspring. Autosomal Minutes appeared in different families in one and five offspring. Two raspberry mutants were found in one family when they were tested first as *v f car* crossovers. A dominant notching of the wings and a suppressor of forked (to be described below) appeared once each. Use of induced mutations has previously been made by HARRIS (1929) to show that male germ cells treated during late stages of their development are used up as sperm within 12 days, after which time the active sperm are derived from cells treated in spermatogonial stages. The gonial origin of such random changes as three of these mutations is hardly open to question. Consequently, the mutations serve as measures of what cells were gonial at the time of treatment. Only the fact of spontaneous crossing over in *c3G* females prevents the ascribing of all the recombinations of the last three cultures to oögonial exchanges.

The possible gonial nature of some or all of the balance of the recombinations may be obscured in at least two ways. Gonial crossing over may occur too late for its products to include two adjacent eggs and the inter-

vening nurse cells, or it may occur in a female whose offspring are few in comparison with the number of egg strings in her ovaries. The first possibility is difficult to investigate, but the effect of size of family upon the recovery of several crossovers of gonial origin may be indicated in table 4.

TABLE 4  
*Distribution of crossovers among families of different sizes.*

| NO. OFFSPRING<br>PER FAMILY | NO. FAMILIES | CROSSEOVERS FOUND |          |
|-----------------------------|--------------|-------------------|----------|
|                             |              | SINGLY            | PLURALLY |
| 1-25                        | 31           | —                 | —        |
| 26-50                       | 20           | 2                 | —        |
| 51-75                       | 27           | 1                 | 1        |
| 76-100                      | 12           | —                 | —        |
| 101-125                     | 13           | 4                 | —        |
| 126-150                     | 11           | —                 | 1        |
| 151-175                     | 6            | —                 | —        |
| 176-200                     | 5            | —                 | 1        |
| 201-225                     | 2            | —                 | —        |
| 226-250                     | 6            | —                 | 1        |

Total families: 133. Average number of offspring: 81.4.

Median number of offspring: c. 65.

Most of the oögonial crossovers were found in families larger than those in which the solitary ones were recovered, and all of the clustered ones were in families larger than the median size. Conversely, three of the singly occurring recombinations appeared in families of 64, 44, and 33 offspring. These numbers were barely large enough to include two eggs from each of the egg strings, of which BERGNER (1928) reports about 30 per normal female.

Although the distribution of crossovers in this experiment was different from that found by GOWEN (1933), the frequency of exchange was about the same. Our frequency of recombinations in the X chromosome was  $1/677$  and the estimated frequency of crossing over was  $1/985$ . GOWEN's frequency of recombination (and of crossing over) was  $1/612$  without the use of X-rays.

Several interesting classes other than recombinations appeared in our experiment, due mainly to the well known effects of the asynaptic factor. Among the 10,000 offspring 158 triploids, 32 haplo-IV flies including one crossover, 10 intersexes, 3 mosaics and 2 gynandromorphs were recorded. One of the last was triploid and wild type on the head and one side and intersex and *sc cv f* on the other half of the thorax and abdomen. The other was a matroclinous female which had lost one sex chromosome and was *ec ct car* male on the head and one-half of the body.

A new mutation named "suppressor of forked" and given the symbol *su-f* was found while verifying what appeared to be a double crossover vermilion fly. It has been located a little to the right of carnation by crossover tests, and the salivary chromosomes appear to be normal in this region. The phenotype of *f su-f* males and of homozygous females often shows a trace of forked on one bristle, like the character hooked.

## DISCUSSION

In order to relate this new kind of crossing over to the several other kinds, some of which are also but recently recognized, a classification of them is deemed advisable. One is presented in table 5 in which current terminology is used as far as possible. The term somatic crossing over is reserved in its restricted use for the process when it occurs in the soma, and another term, mitotic crossing over, is proposed to include both the somatic and germinal (spermatogonial and oögonial) exchanges taking place before the maturation divisions. These three kinds of mitotic crossing over are in contrast to the well known meiotic, or oöcytial, exchanges.

TABLE 5

*Classification and comparison of different kinds of crossing over in Drosophila melanogaster.*

| KINDS                     | MITOTIC  |                            |          | MEIOTIC                                    |
|---------------------------|--|----------------------------|----------|--|
|                           | SOMATIC  | SPERMATOGONIAL             | OÖGONIAL | OÖCYTIAL                                   |
| Spontaneous occurrences   | rarely   | rarely (X and Y)           |          | customarily                                |
| Frequency altered by      | <div style="display: inline-block; vertical-align: middle;"> <div style="font-size: 3em; vertical-align: middle; margin-right: 5px;">{</div> <div> X-rays<br/>high temperature<br/><br/>Minute factors </div> </div> | X-rays<br>high temperature | X-rays   | X-rays<br>low and high temperatures<br>age |
| Region of greatest change | spindle attachment   | spindle attachment         |          | spindle attachment                         |

Thus the primary classification is made upon the basis of the division process involved, and the subdivisions are based upon the cells in which crossing over occurs. That this is a natural classification is brought out by the comparisons included in table 5, where provisionally all the information about germinal crossing over in normal females, treated or untreated, is considered to be entirely due to meiotic crossing over.

From the comparisons of different kinds of crossing over there are indications that the results which have in the past been obtained by subjecting normal females to X-rays or to temperature extremes may be due to crossovers from two sources, gonial and meiotic, rather than only



from the latter. The crossovers induced in normal females resemble the spontaneous meiotic crossovers less than they resemble those induced in somatic or spermatogonial cells. This is true in respect to the regions affected, the time of formation and the activating agents. If further studies on c<sub>3</sub>G females should show that oögonial crossing over is most frequent in the vicinity of spindle attachments, then it would be reasonable to suppose that a few occurrences of crossing over in early oögonial cells are responsible for most of the increased percentage of recombination offspring. Spontaneous crossing over in oögonial cells might also be the explanation of the unusual distribution exhibited by certain rare crossovers, different classes in different progenies, as noted by SEREBROVSKY (1927), BRIDGES and others.

#### SUMMARY

1. A 2500 r dose of X-rays does not affect the action of the asynaptic factor, c<sub>3</sub>G, on meiotic crossing over between the sex chromosomes of females.

2. In oögonial cells crossing over may occur during irradiation of c<sub>3</sub>G females as shown by the appearance of recombination offspring in unusual clusters from eggs laid ten or more days after treatment. Similarly, groups of identical mutations accompany the clustered crossovers temporally.

3. A classification of the kinds of crossing over into meiotic and mitotic, the latter divided into somatic, spermatogonial and oögonial crossing over, seems justified by the likenesses and differences which are pointed out among them.

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# TRANSLOCATIONS IN MAIZE INVOLVING CHROMOSOME 9

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Received February 4, 1938

## INTRODUCTION

THE *C wx* linkage group associated with chromosome 9 was one of the first to be established in maize. This was largely due to the excellence of the two genes *C* and *wx* as working tools. EMERSON, BEADLE and FRASER (1935) list 28 genes in this group. Nearly all of these are to the left of *wx* or within 10 or 15 units to the right. McCLINTOCK (1930, 1931), CREIGHTON and McCLINTOCK (1931), CREIGHTON (1934) and BURNHAM (1930, 1934a, b) have shown that the gene  $\gamma_2$  is near the end of the short arm of chromosome 9 and *wx* nearer the spindle attachment but still on the short arm. Virescent<sub>1</sub> ( $v_1$ ) is probably on the long arm not far from the spindle attachment (BEADLE 1932, BURNHAM 1934b). The greater portion of the long arm is conspicuous by its lack of definitely known genes. This lack of known genes may be largely due to the difficulty of detecting genes in the distal part of the long arm by means of ordinary linkage tests with such genes as *C*, *sh* and *wx*. Or it may be that such regions are redundant sections of chromosome materials which are also represented elsewhere in the chromosome complement.

Chromosomal interchanges or reciprocal translocations are excellent tools for the exploration of portions of chromosomes where no known genes are available. For most cases it is only necessary to cross any new or unplaced gene with the appropriate translocation stock, backcross with the new gene if recessive or with a normal stock if dominant. Classification of the gene character under consideration and semisterility will give a direct linkage test with a known point on the chromosome.

The chromosome 9 translocations studied by the writer have all been on the long arm, mostly in the region beyond the present known genes. They do not add appreciably to our knowledge of the region covered by the present linkage maps, but do furnish excellent means for the placement and mapping of genes in the extensive region of the long arm where no genes are as yet available.

## PREVIOUS DATA ON TRANSLOCATIONS INVOLVING CHROMOSOME 9

T8-9a (BURNHAM 1930, 1934b; McCLINTOCK 1930, 1931; CREIGHTON and McCLINTOCK 1931; BEADLE 1932). The interchange on chromosome 9 on the long arm .4 of distance out from spindle attachment. Linkage order *C-wx-T* with 13.7 percent of crossing-over with *waxy*.

- T5-9a (BURNHAM 1934a). Interchange in proximal part of the short arm of chromosome 9. Linkage order *sh-wx*-T with about two percent of crossing over with waxy. Much non-homologous pairing and much suppression of crossing over.
- T6-9a (ANDERSON 1934, MCCLINTOCK 1934). Interchange about one-third the way out on the long arm of chromosome 9. Linkage order *C-wx*-T with 11.6 percent of crossing over with waxy.

#### TRANSLOCATIONS INCLUDED IN THE PRESENT STUDY

In the present paper, data are presented on the following translocations which are included in the writer's published list (ANDERSON, 1935); T 1-9a, b, c, 2-9a, b, 3-9a, b, 4-9a, b, 6-9a, b, and 8-9b. In addition two others are included which will be designated T3-9c and T9-10b. Both were from the writer's X-rayed material (ANDERSON 1935, Lot 1, sixty minute treatment, and Lot 2, fifty minute treatment, respectively). The chromosomes involved were determined by diakinesis observations of intercrosses with known translocations. T3-9c gave two rings of four in crosses with T 1-2a, T 1-7a, T 4-8a, T 5-7a and T 4-5a, and a ring of six with T 8-9a and T 2-3a. T 9-10b gave two rings of four with T 1-7a, T 4-8a, T 4-5a, and T 2-3a, and a ring of six with T 3-9a and T 3-10a. These determinations of chromosomes involved have been checked by cytological observation at mid-prophase and by linkage tests.

No data are presented on T 9-10a as this translocation involves problems connected with the survival of unbalanced gametes and will be reported as a separate paper.

#### LINKAGE DATA

Since most of the linkage tests with genes from chromosome 9 were made involving the same genes in the same linkage order, the summarized data have been combined in a few tables. The individual cultures have been fairly consistent with the exception of T 4-9a and T 6-9a which are presented in more detail. Except for T 6-9a, backcrosses using the  $F_1$  as male or female have given similar results and are combined in the tables. The arrangement of data in the tables is like that in the linkage summary of EMERSON, BEADLE and FRASER (1935).

Backcross data involving various translocations with the two genes *C* and *wx* are presented in table 1. Data similarly involving the three genes *C*, *sh* and *wx* are presented in table 2. Additional data involving only *wx* are given in table 3 while table 4 gives data involving the genes *C* and *sh* with T 8-9b. In several of the backcross cultures there were large discrepancies between several of the contrary classes, due partly to lower viability of *sh* and *wx*, but chiefly to the presence of  $y_{g_2}$  and one other weak

TABLE 1  
*Backcross progenies from combinations*  $\frac{C+T}{+wx+}$

|         | PARENTAL<br>COMBINATIONS |     | RECOMBINATIONS |    |          |    |              |    |       |      | PERCENT<br>RECOMBINATION COIN-<br>CIDENCE |      |  |  |
|---------|--------------------------|-----|----------------|----|----------|----|--------------|----|-------|------|---|------|--|--|
|         |                          |     | REGION 1       |    | REGION 2 |    | REGIONS 1, 2 |    | TOTAL | I    |   |      |  |  |
|         |                          |     |                |    |          |    |              |    |       | c-wx | wx-T                                      |      |  |  |
| T 1- ga | 154                      | 130 | 50             | 45 | 36       | 7  | 1            | 4  | 427   | 23.4 | 11.2                                      | .44  |  |  |
| T 1- gb | 99                       | 68  | 34             | 30 | 76       | 56 | 14           | 15 | 392   | 23.7 | 41.1                                      | .76  |  |  |
| T 1- gc | 74                       | 71  | 24             | 38 | 22       | 1  | 6            | 1  | 237   | 29.1 | 12.7                                      | .80  |  |  |
| T 2- ga | 42                       | 47  | 15             | 19 | 27       | 20 | 9            | 12 | 191   | 28.8 | 35.6                                      | 1.07 |  |  |
| T 2- gb | 219                      | 225 | 54             | 83 | 22       | 20 | 2            | 3  | 628   | 22.6 | 7.5                                       | .47  |  |  |
| 3- ga   | 116                      | 55  | 29             | 8  | 5        | 2  | —            | —  | 215   | 17.2 | 3.3                                       | —    |  |  |
| 3- gb   | 127                      | 148 | 45             | 50 | 10       | 11 | 3            | 3  | 397   | 25.4 | 6.8                                       | .87  |  |  |
| 3- gc   | 65                       | 65  | 15             | 25 | 14       | 2  | —            | 1  | 187   | 21.9 | 9.1                                       | .27  |  |  |
| 4- ga   | 76                       | 68  | 18             | 43 | 29       | 40 | 9            | 14 | 297   | 28.3 | 31.0                                      | .88  |  |  |
| 4- ga   | 134                      | 123 | 46             | 51 | 24       | 16 | 1            | 5  | 400   | 25.7 | 11.5                                      | .51  |  |  |
| 6- gb   | 238                      | 220 | 41             | 62 | 15       | 4  | —            | 3  | 583   | 18.2 | 3.8                                       | .75  |  |  |
| 8- gb   | 31                       | 31  | 9              | 11 | 25       | 14 | 4            | 6  | 131   | 22.9 | 37.4                                      | .89  |  |  |
| 9-10b   | 175                      | 126 | 67             | 77 | 14       | 12 | 1            | —  | 472   | 30.7 | 15.7                                      | .12  |  |  |

chlorophyll gene in some of the *c wx* stocks used. Under somewhat unfavorable weather conditions these proved practically lethal. While theoretically a lethal gene should not alter the linkage percentages, data in-

TABLE 2  
*Backcross progenies from combinations*  $\frac{C+}{+sh\ wx+} \frac{+T}{+}$

|        | PARENTAL<br>COMBINA-<br>TIONS |    |   | RECOMBINATIONS |    |          |    |          |   |         |   |         |   |         |   | TOTAL |               |
|--------|-------------------------------|----|---|----------------|----|----------|----|----------|---|---------|---|---------|---|---------|---|-------|---------------|
|        |                               |    |   | REGION 1       |    | REGION 2 |    | REGION 3 |   | 1 AND 2 |   | 1 AND 3 |   | 2 AND 3 |   |       | 1, 2<br>AND 3 |
|        |                               |    |   |                |    |          |    |          |   |         |   |         |   |         |   |       |               |
| T 1-gb | 132                           | 69 | 2 | 7              | 25 | 25       | 66 | 49       |   |         | 2 | 2       | 9 | 8       | 1 | 397   |               |
| 1-gc   | 145                           | 41 | 3 | 3              | 28 | 29       | 24 | 5        | 1 |         |   |         | 1 | 3       |   | 283   |               |
| 2-ga   | 42                            | 34 | 3 | 1              | 13 | 15       | 19 | 6        |   |         |   |         | 3 | 3       | — | 139   |               |
| 4-ga   | 57                            | 21 | 1 | 2              | 17 | 15       | 8  | 2        | 1 |         |   |         | 2 | 3       |   | 129   |               |
| 4-gb   | 68                            | 78 | 6 | 1              | 19 | 14       | 1  | 4        | 1 |         |   |         | 1 |         |   | 193   |               |

TABLE 3  
*Backcross progenies from combinations*  $\frac{+T}{wx+}$ .

|      | PARENTAL COMBINATIONS |     | RECOMBINATIONS |    | TOTAL | PERCENT RECOMBINATIONS |  |
|------|-----------------------|-----|----------------|----|-------|------------------------|--|
|      |                       |     |                |    |       |                        |  |
| 2-9a | 63                    | 56  | 31             | 25 | 175   | 32.0                   |  |
| 3-9a | 202                   | 176 | 9              | 6  | 393   | 3.8                    |  |
| 3-9c | 67                    | 66  | 6              | 2  | 141   | 5.7                    |  |

TABLE 4  
*Backcross progeny from*  $\frac{C+T}{+wx+}$ .

| PARENTAL<br>COMBINATIONS |    | RECOMBINATIONS |   |          |    |              |   | TOTAL | PERCENT<br>RECOMBINATION |             |      |
|--------------------------|----|----------------|---|----------|----|--------------|---|-------|--------------------------|-------------|------|
|                          |    | REGION 1       |   | REGION 2 |    | REGIONS 1, 2 |   |       | <i>c-sh</i>              | <i>sh-T</i> |      |
|                          |    |                |   |          |    |              |   |       |                          |             |      |
| T 809b                   | 65 | 55             | 1 | 3        | 28 | 34           | 2 | 1     | 189                      | 3.7         | 34.4 |

volving a lethal gene are not entirely trustworthy for accurate determinations. But for the approximations required in the present work, the data are probably accurate enough.

The T 4-9a progenies fall into two sharply different groups. Three cultures from a single plant, 11-315-2, gave 10.2, 12.2, and 11.6 percent crossing over for the *wx-T* interval. Two cultures from sibs of this plant gave 27.1 and 34.9 percent for the same region. A closely related plant gave 27.6 percent. The two groups of data have been summarized separately. Cytological observations of one plant at mid-prophase of meiosis has shown the interchange of chromosomes about four fifths the way out on the long arm of 9 and very near the spindle attachment on 4. This position would give an expectation of loose linkage corresponding to the higher crossover value. The low value obtained in cultures from 11-315-2 might be due to the presence of some other chromosomal alteration such as the short inversion about the spindle attachment of chromosome 4 which is known to be present in some stocks (McCLINTOCK 1933).

Some further data have been obtained on T 6-9a. Two sister  $F_1$  plants were backcrossed reciprocally with a *c wx* stock. The data are given in table 5. With such small numbers of plants involved, not much reliance can be placed on these data. There is a marked difference in the crossover values when the plants are used as female or male, but the difference is opposite to that previously found for the T -Y -Pl region of chromosome 6 (ANDERSON 1934). Obviously the crossing-over behavior of T 6-9a needs to be checked more carefully.

TABLE 5  
*Reciprocal backcrosses of T 6-9a. Parental formula  $\frac{C+T}{+wx+}$ .*

| F <sub>1</sub> PARENT | F <sub>1</sub> USED AS | PARENTAL COMBINATIONS |     | RECOMBINATIONS |    |          |   |              |   |     | TOTALS | PERCENT CROSSING OVER   |  |
|-----------------------|------------------------|-----------------------|-----|----------------|----|----------|---|--------------|---|-----|--------|-------------------------|--|
|                       |                        |                       |     | REGION 1       |    | REGION 2 |   | REGIONS 1, 2 |   |     |        | <i>C-wx</i> <i>wx-T</i> |  |
|                       |                        |                       |     |                |    |          |   |              |   |     |        |                         |  |
| 11-318-1              | ♀                      | 18                    | 20  | 8              | 9  | 6        | 7 | 2            | 0 | 70  | 27.1   | 21.4                    |  |
|                       | ♂                      | 108                   | 107 | 26             | 45 | 9        | 2 | 1            | 2 | 300 | 24.7   | 4.7                     |  |
| 11-318-5              | ♀                      | 51                    | 52  | 35             | 24 | 4        | 9 | 5            | 3 | 183 | 36.6   | 11.5                    |  |
|                       | ♂                      | 78                    | 17  | 12             | 12 | 3        | 2 | 2            | 1 | 127 | 21.3   | 6.3                     |  |

## CYTOLOGY

Cytological examinations have been made of aceto-carmin preparations at mid-prophase of meiosis. Measurements and estimates of position of the interchange of chromosomes have been made from camera lucida tracings of the clearest figures. The position of the interchange is recorded in tenths of the distance from the spindle attachment to the end of the arm. Thus 9 L.4 indicates chromosome 9 long arm four-tenths of distance from the spindle attachment. In general these placements are probably reliable to about .1 or .2 of the length of the arm. Those near the end of the chromosome are somewhat less reliable. In some cases, notably T 5-9a (BURNHAM 1934a) and T 6-9b, there is much non-homologous pairing (McCLINTOCK 1933), which makes it difficult to determine the normal position of the interchange.

The three translocations T 8-9a, T6-9a, and T 5-9a have been described. The following have been examined cytologically:

|         |        |              |
|---------|--------|--------------|
| T 1-9b  | 1 L .6 | 9 L .5       |
| T 1-9c  | 1 S .6 | 9 L .2 +     |
| T 2-9a  | 2 S .7 | 9 L .6 +     |
| T 2-9b  | 2 S .1 | 9 L .2       |
| T 3-9a  | —      | 9 L .1 +     |
| T 3-9c  | 3 L .1 | 9 L .2       |
| T 4-9a  | 4 L .1 | 9 L .8       |
| T 4-9b  | 4 L .6 | 9 L .2       |
| T 8-9b  | 8 S .2 | 9 L .8       |
| T 9-10b | 9 L .3 | 10 near s.a. |

T 1-9a and T 3-9b have not been studied. T 6-9b shows much non-homologous pairing, but seems to have the interchange in the proximal part of the long arm of 9.

The writer is indebted to Mrs. GERTRUDE G. FRANDSEN for most of the cytological preparations.

## RELATION OF POSITION OF INTERCHANGE TO LINKAGE WITH WAXY

TABLE 6

*Comparison of position of interchange with map distance from waxy.*

|        | CYTOLOGICAL<br>POSITION | PERCENT CROSSING<br>OVER FROM <i>wx</i> | NUMBER OF PLANTS<br>IN LINKAGE TESTS |
|--------|-------------------------|---|--------------------------------------|
| T 5-9a | S .1                    | 2.0                                     | (BURNHAM 1934a)                      |
| 3-9a   | L .1+                   | 3.6                                     | 608                                  |
| 4-9b   | L .2                    | 3.1                                     | 193                                  |
| 6-9b   |                         | 3.8                                     | 583                                  |
| 3-9b   |                         | 6.8                                     | 397                                  |
| 2-9b   | L .2                    | 7.5                                     | 628                                  |
| 3-9c   | L .2                    | 7.6                                     | 328                                  |
| 1-9c   | L .2+                   | 12.1                                    | 520                                  |
| 9-10b  | L .3                    | 5.7                                     | 472                                  |
| 6-9a   | L .3                    | 9.4                                     | 955                                  |
| 1-9a   |                         | 11.2                                    | 427                                  |
| 8-9a   | L .4                    | 13.7                                    | (BURNHAM 1934b)                      |
| 1-9b   | L .5                    | 37.7                                    | 789                                  |
| 2-9a   | L .6+                   | 30.7                                    | 505                                  |
| 4-9a   | L .8                    | 31.0                                    | 297                                  |
|        |                         | 11.5                                    | 529                                  |
| 8-9b   | L .8                    | 37.4                                    | 131                                  |

Table 6 gives a list of the interchanges involving chromosome 9 arranged in approximate order of their position in the chromosome and of their crossover distance from *waxy*. The first column gives the observed position in chromosome 9. The second column gives the percent of crossing over from *waxy*. The third column gives the total number of backcross plants on which the crossover percentages are based, summarized from the preceding tables. The correlation between the cytological observations and the percentages of crossing over is very close, considering the probable amount of error in the cytological placements. The loose linkages shown by T 1-9b, T 2-9a, T 4-9a, and T 8-9b probably represent longer map distances, which can be studied more effectively when one or more helpful genes are found in the distal part of chromosome 9.

## SUMMARY

Data are presented on linkage relations with *waxy* for fourteen translocations involving the long arm of chromosome 9.

The amount of crossing over with *waxy* is closely correlated with the cytological position of the interchange (table 6).

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# THE PRODUCTION OF HOMOZYGOUS DEFICIENT TISSUES WITH MUTANT CHARACTERISTICS BY MEANS OF THE ABERRANT MITOTIC BEHAVIOR OF RING-SHAPED CHROMOSOMES\*

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Received February 25, 1938

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## I. INTRODUCTION

IT IS the purpose of this paper to describe the method by which viable tissues, homozygous deficient for a known region of a chromosome, may be produced in maize. The chromosomal region involved includes the locus of the gene *Bm 1* in chromosome V (allele of *bm 1*, brown mid-rib, producing a brown color in the lignified cell walls). The lignified cell walls of the homozygous deficient tissue exhibit the features characteristic of the known recessive gene *bm 1* although the locus of this gene is absent.

The method of obtaining the homozygous deficient tissue is related to the unique behavior of ring-shaped chromosomes during somatic mitosis. This behavior has been briefly mentioned in previous publications (McCLINTOCK 1932; RHOADES and McCLINTOCK 1935). Ring-shaped chromosomes do not always maintain themselves unaltered through successive nuclear cycles in the maize plant. They may (1) increase in size through duplication and reduplication of segments of the original ring, (2) decrease in size by deletions of segments from the ring, (3) be totally lost from the nuclei or (4) be present in increased numbers in the different nuclei. Whatever the method by which a change in size occurs, only ring chromosomes are produced from ring chromosomes..

In maize it has been found that deficiencies in certain regions of the

\* The cost of the half-tone illustrations in this paper is met by the Galton and Mendel Memorial Fund.

<sup>1</sup> Contribution from the Department of Botany in cooperation with the Department of Field Crops, Genetics Research Project, Missouri Agricultural Experiment Station, Journal Series No. 570.

chromosomes may be transmitted successfully through the egg but not through the pollen (BURNHAM 1932; STADLER 1935). Pollen possessing a deficient chromosome plus a ring-shaped fragment chromosome should be functional if the ring-shaped fragment completely compensates for the deficiency. By utilizing a deficiency transmissible through the eggs and rendered non-lethal in the pollen by the inclusion of a ring fragment covering the deficiency, a zygote with two deficient chromosomes plus a ring chromosome can be produced. This zygote is heterozygous for the

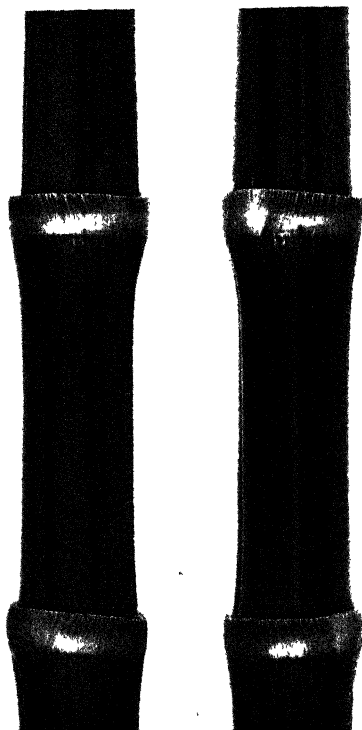


FIGURE 1.—Two sides of a stalk of a variegated plant. The leaves at the two nodes have been removed. The dark bands are *bm1*, the light bands, *Bm1*.

deficiency. This heterozygosity in the resulting individual would be maintained as long as an unaltered ring chromosome was present. Should the ring chromosome be lost in subsequent nuclear divisions, or should it change in size through loss of a segment within it, the tissues arising after such loss or alteration would be homozygous deficient for the entire deficiency in the first case or for regions within the limits of the deficiency in the second case.

Two cases of deficient rod chromosomes with complementary ring chromosomes were available for this study. The two cases arose in the

progeny of X-rayed pollen containing a normal haploid complement with the dominant gene *Bm 1*. This pollen, when placed upon silks of *bm 1* plants with a normal chromosome complement, gave rise, among a progeny of 466, to two individuals which were variegated for *Bm 1* and *bm 1* (figure 1). Aberrant behavior of a ring chromosome produced by the X-ray treatment and carrying the gene *Bm 1* was suspected to be the cause of the variegation.

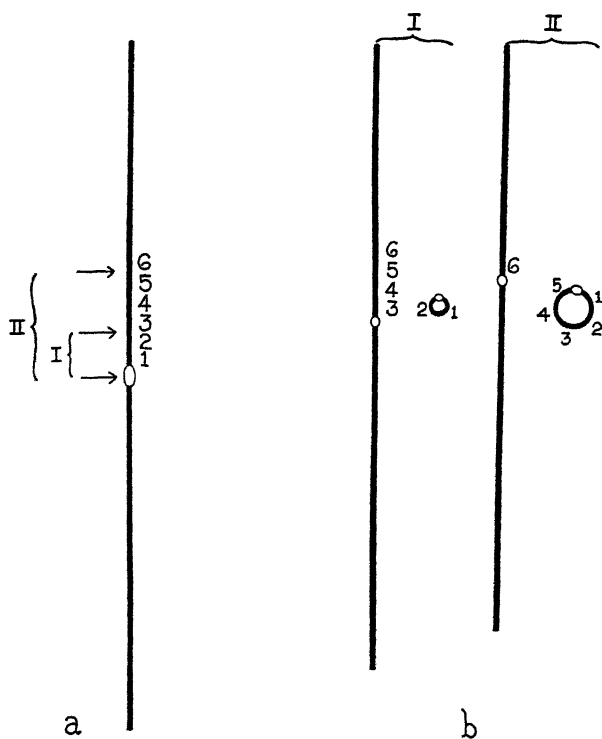


FIGURE 2.—a. Diagram of a normal chromosome V. The slightly bulging section represents the spindle fiber attachment region. The sets of arrows, I and II, point to the positions of breaks which gave rise to the two deficient rod chromosomes and their compensating ring chromosomes illustrated in I and II of b. The deficient rod and compensating ring chromosomes of I are referred to in the text as Def 1 and R 1 respectively, those of II as Def 2 and R 2 respectively

Examination of synaptic configurations in sporocytes revealed not only the presence of a small ring-shaped chromosome in each plant but also a deficiency in one chromosome V. In each case, the size of the ring-shaped chromosome and the extent of the deficiency in the rod-chromosome were comparable. The deficiency in both cases involved a section of the short arm immediately adjacent to the spindle fiber attachment region. Since the ring fragment in both cases possessed a small but definite spindle fiber attachment region, these regions being clearly visible in meiotic prophase,

it was assumed that in each case the deficient rod and its compensating ring chromosome arose as the result of two breaks in the normal chromosome V, one break passing through the spindle fiber attachment region, the other breaking the chromosome at a distance from the spindle fiber attachment region equal to approximately  $1/20$  (Case I) and  $1/7$  (Case II) of the total length of chromosome V (figure 2). Fusions two by two of the broken ends resulted in a deficient rod and a compensating ring chromosome each with a section of the original spindle fiber attachment region. Since both the deficient rod and the ring chromosome possessed a section of the spindle fiber attachment region, both could be maintained through nuclear cycles.

✓ Proof that the ring chromosome represented the region for which the rod chromosome was deficient was furnished by the synaptic configurations produced by homologous associations of the three chromosomes: the normal chromosome V contributed by the female parent, the deficient rod chromosome V and the small ring chromosome contributed by the male parent (figures 25 and 26 and photographs of the same, 17 and 18, Plate II). Cytological examination of different portions of the tassel disclosed the loss of the ring chromosome in several branches. Similarly, within a single anther, groups of cells were found lacking the ring chromosome. It was suspected, therefore, that the ring chromosome carried the locus of *Bm 1*, its loss during somatic mitoses being responsible for the presence of the *bm 1* (brown) streaks in these plants. Conclusive proof for this was derived from the progeny of these two plants when crossed to normal *bm 1* plants. The progeny included variegated (*Bm 1* and *bm 1*) and *bm 1* plants. Of the variegated plants, microsporocytes of 148 individuals were examined for the presence of the ring chromosome. The ring fragment was found in 146 of these individuals although in many plants several branches of the tassel lacked the ring fragment. In two plants no ring chromosome was found in the several branches of the tassel which were collected. Of the totally *bm 1* plants, 47 were examined. In no case was a ring chromosome found. In a *bm 1* tiller of a variegated plant, a considerably reduced ring chromosome was found. It is probable in this case that the *Bm 1* locus had been deleted from the ring chromosome through somatic alterations to be described in the next section. Individual collections were made on the two sides of plants which were approximately half *bm 1* and half variegated. In these cases, the presence of the ring chromosome could be established only on the variegated side.

## II. THE MITOTIC BEHAVIOR OF RING-SHAPED CHROMOSOMES

The interpretation of the variegation and of the production of homozygous deficient tissues has been based on a knowledge of the behavior of

ring-shaped chromosomes in somatic nuclear cycles. A description of what has been observed regarding the appearance and behavior of the ring chromosomes in meristematic regions is therefore necessary before the individual cases can be considered. Although the primary cause of irregularities in the nuclear cycles is undoubtedly the same for large and small ring-shaped chromosomes, the subsequent behavior and the genetical consequences vary in these two extremes. The behavior of large ring-shaped chromosomes will be considered first; this will be followed by an account of the small ring-shaped chromosomes; finally, correlations and conclusions will be drawn regarding ring-shaped chromosomes in general.

*Mitotic behavior of large ring-shaped chromosomes*

Since the two ring-shaped chromosomes of cases I and II, figure 2, are both small, a large ring-shaped chromosome originally representing most of chromosome II has been examined (McCLINTOCK 1932). The observations were made on longitudinal sections of actively growing root tips. Observations at meiotic prophase in this plant had clearly indicated that changes in size and hence chromatin content of the ring chromosome were occurring in the premeiotic nuclei. Groups of related cells usually had similar ring chromosomes but the differences in unrelated cells were very great. In a few cells the altered ring chromosome was larger than the normal chromosome II. In some cells it had been reduced to only a few chromomeres. All gradations between these two extremes were found in different sporocytes of this same plant. The smallest ring chromosome has obviously undergone a great loss of chromatin. The original ring chromosome possessed a single knob. Evidence for duplication of segments other than the obvious increase in size of the ring chromosome was clearly registered in some cells by the increase in the number of knobs. Rings with two, three and four knobs were found.

It was suspected that the alteration in chromatin content of the ring was related to the division cycle of the chromosome. Observations of mitoses in root tip meristems suggested the manner in which the alterations occur without, however, revealing the primary cause. If one assumes that during the splitting process or after the split has occurred, a cross-over took place between the two sister chromatids, a double-sized, continuous ring with two spindle fiber attachment regions would be produced. A second crossover between the two sister chromatids could result in an interlocking of the sister ring chromosomes provided the second crossover did not counteract the first. The presence of double-sized rings with two spindle fiber attachment regions at late anaphase and early telophase was clearly evident in a number of cells (figures 5, 7, 15, 16; photographs 4, 5, 8, Plate I). Unfortunately the presence of interlocking rings could not

be determined directly since the chromosomes of maize in somatic cells are relatively small. Many anaphase figures were suggestive but none could be definitely distinguished from double-sized rings with a twist at the mid-region. From the point of view of the origin of such configurations it would be important to know the relative percentage of each type. From actual counts it is certain that the double-sized rings are present in at least one-third of the aberrant figures. The actual number of late anaphase and early telophase figures with chromatin bridges produced by double-sized or interlocked rings amounted to approximately 8 percent of a total of 1145 figures recorded in roots whose ring chromosome had not materially reduced in size in most of the cells (D, table 1).

TABLE 1

*The frequency of normal and aberrant somatic anaphase and early telophase configurations in plants with different ring chromosomes.*

|   | RING CHROMOSOME          | NORMAL | ABERRANT | % ABERRANT |
|---|--------------------------|--------|----------|------------|
| A | R1                       | 605    | 1        | 0.16       |
| B | R2                       | 1195   | 14       | 1.1        |
| C | R2 plus enlarged R2      | 1169   | 76       | 6.1        |
| D | Large ring chromosome II | 1053   | 92       | 8.1        |

Since the fate of the double-sized or interlocked rings is not the same in all late anaphase and telophase figures, a number of types of behavior from anaphase to late telophase have been diagrammed in figure 3. Representative drawings from different cells are given in figures 5 to 24 and photographs of Plate I. In the diagrams, the behavior of double-sized rings has been emphasized since this type could be clearly recognized in many cells. They are either clearly open or show a twist at the mid-region. Some of the interlocked rings should produce figures resembling those shown in the diagram and would not be easily distinguished from them.

In most of the mitotic cycles the ring chromosome splits along a single plane, separation of the two halves proceeding normally at anaphase, figure 4. In the late anaphase figures the double-sized rings produce a double bridge the chromatin of which is pulled taut (figure 5, photograph 1, Plate I). It is suspected that breakage of the chromatin bridges sometimes occurs during this period (photograph 10, Plate I). Since such figures were not included in the counts mentioned above, the 8 percent of anaphase and telophase figures with bridges represent the minimum number of cells in which double-sized or interlocked rings occurred. Some of the telophase figures suggest an early breakage of one or both strands of the double bridge (figures 8 and 9 and photographs 5 and 6, Plate I).

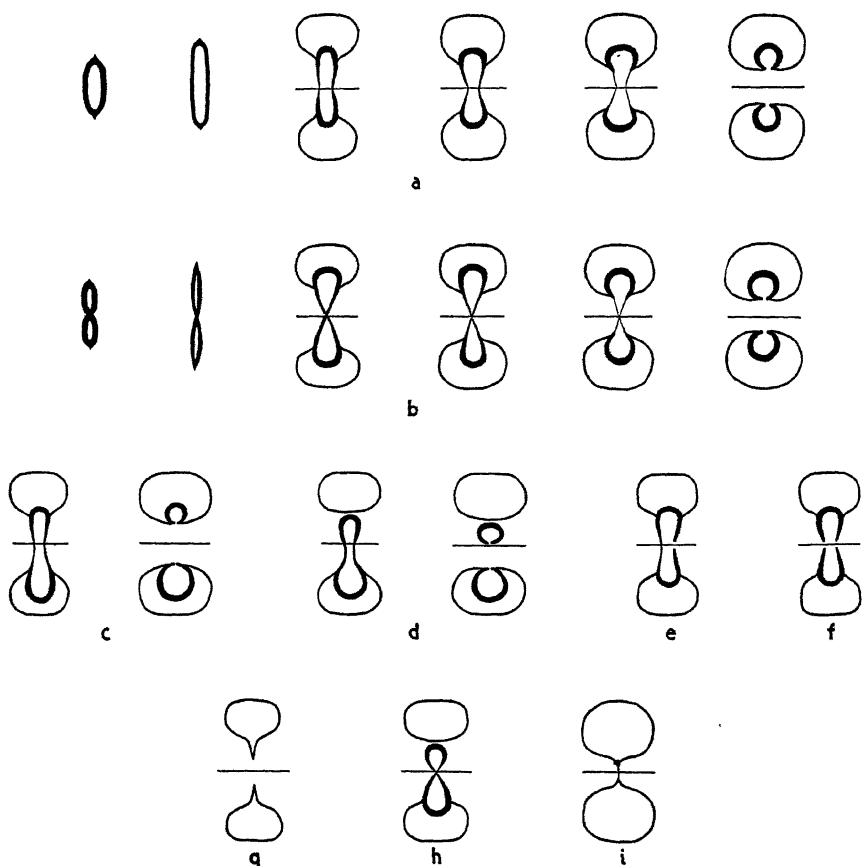


FIGURE 3.—Diagrams illustrating the behavior in somatic mitosis of double-sized ring chromosomes with two spindle fiber attachment regions produced from the two split halves of a single ring chromosome.

a. Successive stages from mid-anaphase to mid-telophase of a medianly placed double-sized ring chromosome. The cell plate determines the positions at which breaks will occur in the two chromatin bridges.

b. Similar to a except that a twist is present in the bridge strands of the double-sized ring chromosome.

c. Appearance in early and mid-telophase of a double-sized ring which was non-medianly placed in the spindle figure. The components entering each daughter nucleus vary in chromosome length and constitution.

d. Similar to c except that the upper portion of the double-sized ring chromosome is not included in the reorganizing telophase nucleus. Such behavior results in the loss of a component of the ring chromosome from one of the daughter nuclei.

e. Appearance at mid-telophase of a double-sized ring chromosome with one broken bridge strand.

f. Appearance at mid-telophase of a double-sized ring chromosome with both strands broken.

g. Appearance at very early telophase suggesting an early breakage of bridge strands of a double-sized ring chromosome (or two interlocked sister ring chromatids).

h. Comparable situation as illustrated in d except that the strands of the bridges are twisted at the cell-plate region.

i. Fine bridge of chromatin between two resting nuclei suggesting that a breaking of the strands had not occurred at telophase.



In many cases, breakage of the strands composing the bridge does not occur at anaphase; compare photographs 9 and 10, Plate I. The moving apart of the spindle fiber attachment regions in the double-sized rings is retarded by the tension of the chromatin bridges. The subsequent behavior is conditioned by the position in the spindle figure of this retarded ring or of two retarded interlocked rings. As the telophase sets in there is an

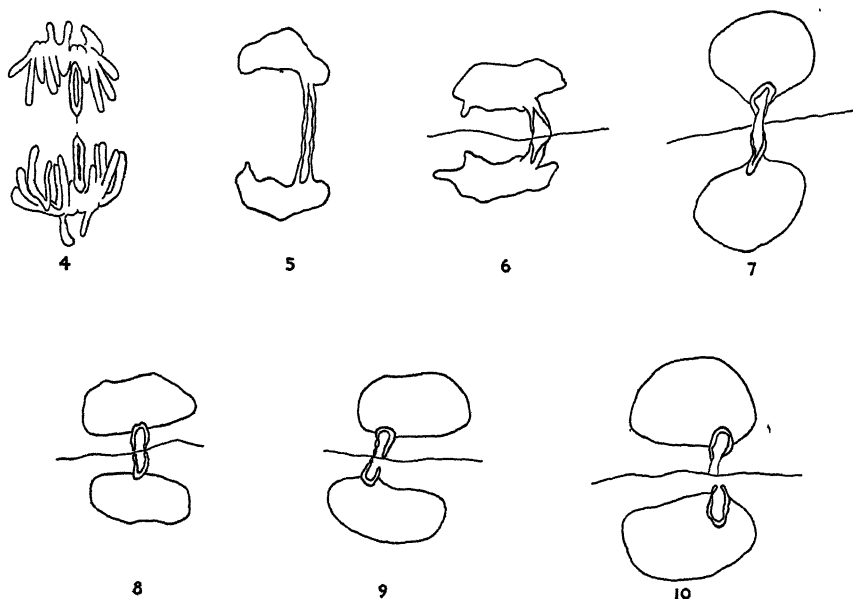


FIGURE 4.—Normal separation of a ring chromosome in somatic anaphase.

FIGURE 5.—A double-sized ring chromosome in early telophase.

FIGURE 6.—A double-sized ring chromosome at a slightly later stage than that shown in figure 5.

FIGURE 7.—Double-sized ring chromosome at mid-telophase. The bridge strands close to the cell-plate have become very thin. The shape of the chromosome within the nuclei has become discernible.

FIGURE 8.—Mid-telophase. Early breakage at the cell-plate region of two bridge strands of the double-sized ring. See comparable figure, photograph 6, Plate I.

FIGURE 9.—Mid-telophase. Early breakage at the cell-plate region of one bridge strand of a double-sized ring. See photograph 5, Plate I.

FIGURE 10.—Late telophase. Breakage of bridge strands of a double-sized ring chromosome at the cell-plate region and withdrawal of the chromatin into the nucleus at the lower part of the figure.

immediate release of tension on the chromatin bridges produced through the swelling of the forming nuclei (photograph 2, Plate I). As the nuclei continue to swell and approach the cell-plate, the chromatin of the ring *within* the nuclei is relaxed, allowing the form of the ring chromosome to be clearly defined (figures 8, 9, 11, 16; photographs 3, 4, 5 and 8, Plate I). At this stage the tension on the chromatin threads from the nuclear mem-

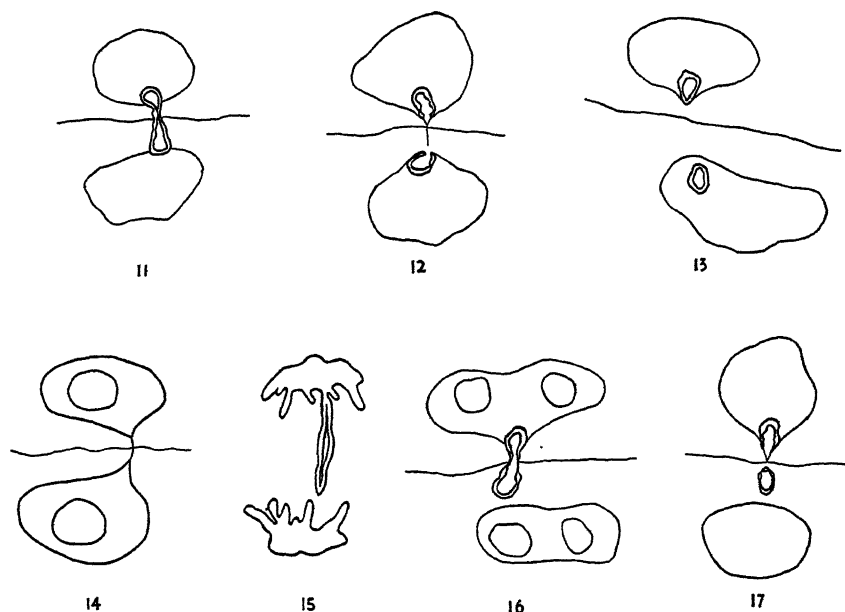


FIGURE 11.—Double-sized ring at mid-telophase. See photograph 3, Plate I.

FIGURE 12.—Similar stage to that shown in figure 10 resulting from a previous double-sized ring with a twist at the mid-region or from two sister ring chromatids which were interlocked.

FIGURE 13.—Sister nuclei at late telophase. The positions of the ring chromosomes suggest a previous bridge formation which has broken.

FIGURE 14.—Resting stage. Sister nuclei with a fine connecting chromatin bridge.

FIGURE 15.—Non-median position of a double-sized ring chromosome at late anaphase. See photograph 7, Plate I. In the photograph there is a twist in the ring chromosome.

FIGURE 16.—Mid-telophase. The result of a non-median placement of a double-sized ring chromosome at anaphase. See photograph of the same, 8, Plate I.

FIGURE 17.—Mid-telophase. The result of a non-median placement of a double-sized ring chromosome with a twist, or of two interlocked sister ring chromatids.

brane to the cell-plate again increases. The threads become thin and taut as if being pulled into the nuclei (figures 7, 16, 17; photograph 8, Plate I). In a few cases, these fine chromatin threads are seen in relatively late telophase nuclei (figure 14). Since they usually do not persist into late stages, breakage must usually occur during the earlier telophase period. There were many sister telophase nuclei observed in which the ring chromosome in each nucleus was close to the region of the nuclear membrane lying nearest the cell-plate (figures 12 and 13). Such figures probably represent the last stage in the progress of the previously double-sized or interlocked rings. It should be emphasized that fusions of broken ends must occur after such breakage, since only ring chromosomes have been found to arise from ring chromosomes although rod chromosomes might be expected.

It sometimes happens that the passage of one spindle fiber attachment

region of a double-sized ring proceeds toward its pole in advance of the opposing spindle fiber region. Consequently, the double-sized ring is not medially placed in the spindle figure. The cell-plate then intercepts the chromatin bridges in a non-median position (figure 15, photograph 7, Plate I). As a result, the components of the double-sized ring entering sister telophase nuclei will be unequal in size and chromatin constitution. One segment of the double-sized ring is sometimes not included in the telophase nucleus on its side of the cell-plate (figures 16 and 17, photograph 8, Plate I).

If the chromosome is not split at anaphase, fusions of broken ends could give rise in the next division to normally disjoining sister ring chromosomes, or if twists are present in the chromonema before fusion, to a continuous double-sized ring or interlocked sister ring chromosomes when

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#### EXPLANATION OF PLATE I

All magnifications are approximately  $\times 1100$ .

Plate I.—Individual cells from longitudinal sections of the growing points of roots. Photographs 1 to 10 are of the large chromosome II ring. Photographs 11 to 14 show an enlarged R2 chromosome. Photographs 15 and 16 are of the normal R2 chromosome.

Photograph 1. Late anaphase. Bridge produced by separation of the split halves of a ring-shaped chromosome which is in the form of a double-sized continuous ring. There is a twist of the strands at the mid-region.

Photograph 2. Early telophase. Beginning of relaxation of tension on the strands of the double bridge.

Photograph 3. Mid-telophase. Complete relaxation of tension on strands of bridge.

Photograph 4. Mid-telophase. Double-sized ring chromosome.

Photograph 5. Mid-telophase. A double-sized ring chromosome. The strand to the right appears to be broken.

Photograph 6. Mid-telophase. A double-sized ring chromosome. Both strands appear to be broken at the cell-plate region.

Photograph 7. Late anaphase. Non-median placement in the spindle figure of a double-sized ring chromosome.

Photograph 8. Mid-telophase. The result of a non-median placement in the spindle figure of a double-sized ring chromosome. The strands adjacent to the cell-plate have become attenuated. The lower segment of the ring chromosome was excluded from the forming nucleus.

Photograph 9. Very early telophase. Chromatin bridge produced by a double-sized ring chromosome with twisted strands, or possibly two interlocked sister ring chromatids.

Photograph 10. Very early telophase. Figures such as this suggest an early breakage of the strands of a double-sized ring chromosome or of interlocked sister ring chromatids.

Photograph 11. Typical late anaphase position of a small ring-shaped chromosome which will be excluded from the reforming telophase nuclei.

Photograph 12. Early telophase. Excluded ring chromosome which was previously non-medianly placed in the spindle figure. The cell-plate has passed below it.

Photograph 13. Late anaphase. Stage in the process of exclusion of two closely associated ring chromosomes.

Photograph 14. Similar to photograph 13.

Photograph 15. Typical late anaphase position of a small ring-shaped chromosome which will be excluded from the telophase nuclei.

Photograph 16. Mid-telophase. The result of a previously excluded ring-shaped chromosome. The cell-plate has passed below the ring.

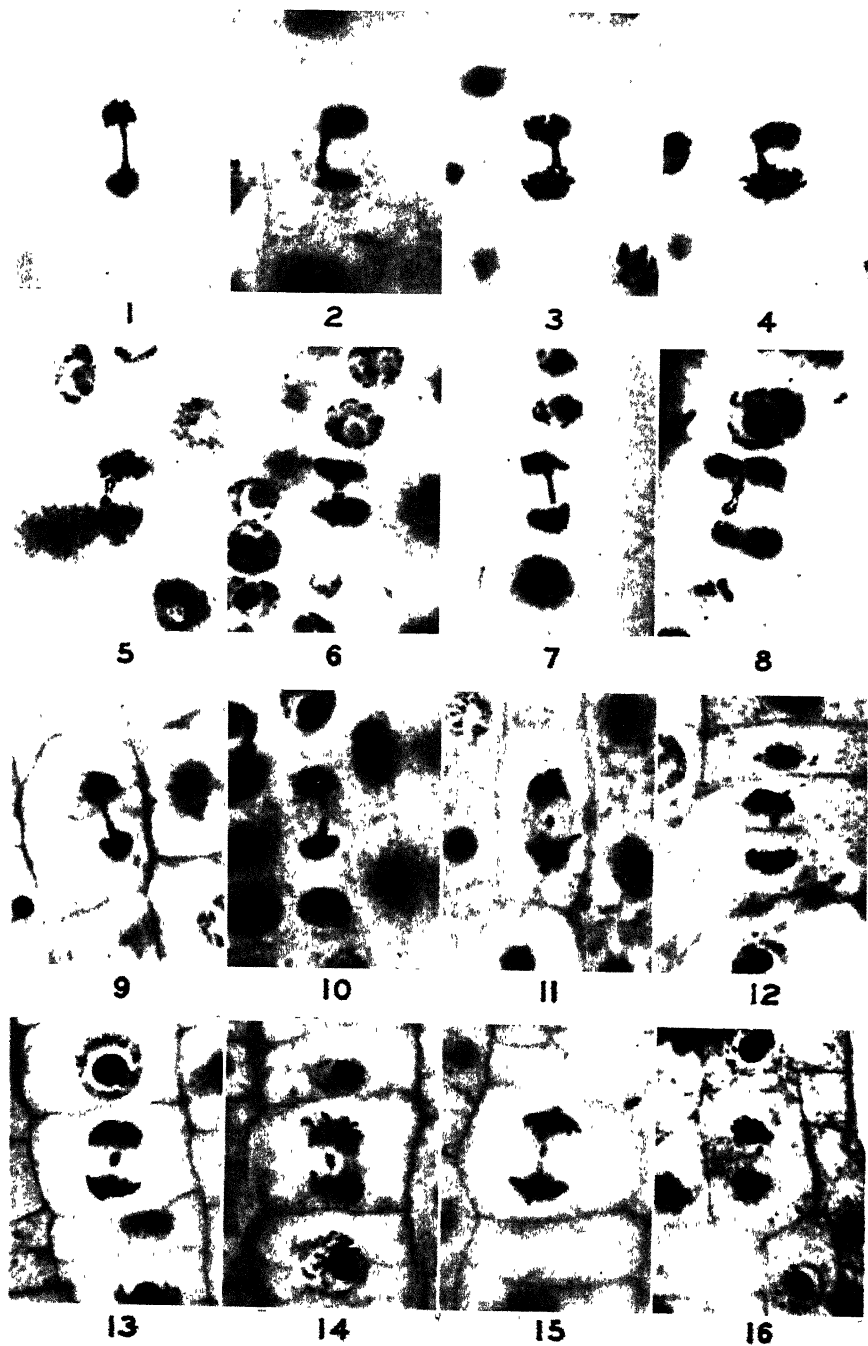


PLATE I

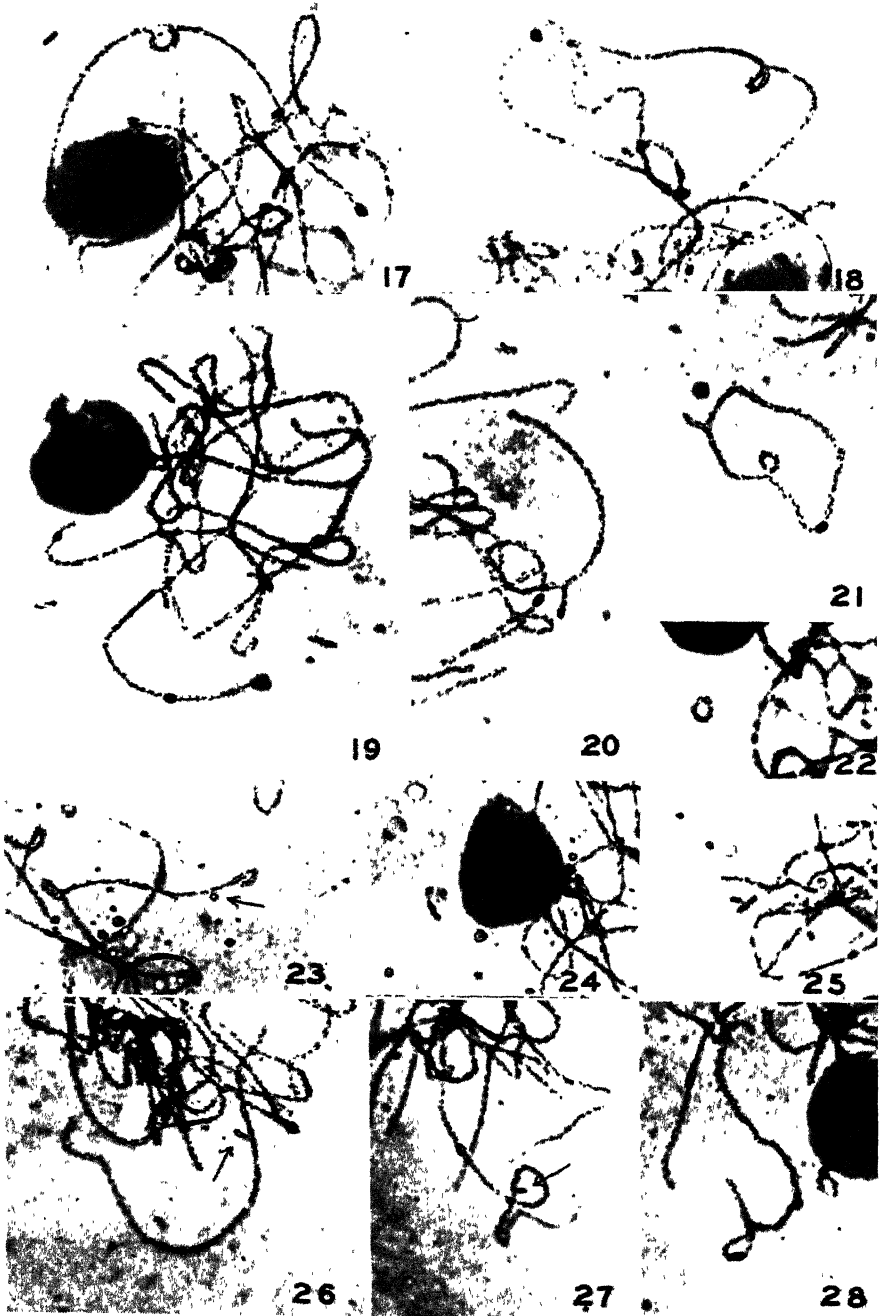


PLATE II

simple assumptions are made regarding the method of splitting or reduplication of a chromonema along a single plane. If the chromosomes are split at anaphase, two by two fusions of the two adjacent broken ends of sister chromatids could result immediately in a continuous double-sized ring. If fusions two by two took place between the non-adjacent broken ends, continuous double-sized rings or interlocked sister ring chromatids could result. If the single (no anaphase split) threads were very much twisted or the double (split present at anaphase) threads coiled about one another, complex configurations would appear in the next anaphase. Only rarely was a figure found suggesting any complexity. If such behavior were the secondary cause of double-sized or interlocked ring chromatids (it cannot be the primary cause, see discussion), adjacent cells in the longitudinal rows could be expected to show chromatin bridges in an appreciable percent of the cases. They were present in a number of longitudinally adjacent cells. However, a very large number of such figures would be necessary to allow a satisfactory statistical study to be made. Although a large number of anaphase and telophase figures with aberrant configurations have been observed, the numbers of these in adjacent cells were insufficient for such a study.

*The mitotic behavior of small ring-shaped chromosomes*

The mitotic behavior of small ring-shaped chromosomes differs from that of large ring-shaped chromosomes in (1) the reduced frequency with

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EXPLANATION OF PLATE II

PLATE II.—All photographs are of pachytene configurations in microsporocytes.  $\times 1100$ .

Photograph 17. Synaptic association of a normal chromosome V, a Def2 chromosome V and an R2 chromosome. See text figure 25.

Photograph 18. Similar to photograph 17. See text figure 26.

Photograph 19. For description, see text figure 27.

Photograph 20. Synaptic association of a normal chromosome V and a Def2 chromosome V. See text figure 28.

Photograph 21. From a sporocyte of a plant with a normal chromosome V, a Def2 chromosome V and two R2 chromosomes. The two rod-chromosomes have associated with one another (note buckle at spindle fiber attachment region). The two ring chromosomes have associated with one another.

Photograph 22. Synaptic association of two R2 chromosomes.

Photograph 23. Late pachytene. The arrow points to the tiny R1 chromosome.

Photograph 24. Collapsed R1 (upper) and R2 (lower) chromosomes associated at their spindle fiber attachment regions.

Photograph 25. Collapsed R1 (left) and R2 (right) chromosomes associated at their spindle fiber attachment regions.

Photograph 26. R1 chromosome (arrow). Its spindle fiber attachment region is stuck to that of bivalent chromosome VIII.

Photograph 27. Collapsed R1 chromosome (arrow) whose spindle fiber attachment region is associated with that of a normal chromosome V bivalent.

Photograph 28. Synaptic association of a normal chromosome V and a Def1 chromosome V. See text figure 30.

which double-sized or interlocked rings arise; (2) the more frequent loss of the ring chromosomes from the nuclei; (3) the considerably less frequent occurrence of changes in size of the ring chromosomes and (4) the occasional increase in the number of rings in a nucleus.

The two small rings in cases I and II, figure 1, have been used to study the behavior of small ring-shaped chromosomes in mitosis. In the subsequent discussions these two ring chromosomes will be referred to as R 1 and R 2 respectively. Cytological examination of the sporocytes in different branches of the tassel in plants with either of these rings had indicated that loss of the ring chromosome was occurring far more frequently than changes in size of the ring. This is in direct contrast to the behavior of large ring chromosomes, where changes in size are more frequent than loss. To obtain evidence on the method of loss, examinations of the meristematic regions of the roots of such plants were made. The tiny R 1 ring chromosome is clearly visible in the prophase nuclei of these cells. However, the description will confine itself to the behavior of the larger of these two rings, R 2, and one of its enlarged derivatives, since anaphases showing aberrant configurations of the R 1 chromosome are found only very rarely.

The aberrant anaphase and telophase configurations are characterized by the median or nearly median position of the double-sized (or interlocked) ring chromosome in the spindle figure (photograph 15 for the normal R 2 and photograph 11 for the enlarged R 2, Plate I). However, they occasionally lie some distance from this position (figure 22 and photograph 12, Plate I). The ring chromosome in these configurations, as with the large ring chromosomes, frequently appears to be double-sized. In roots in which most of the nuclei contained the normal R 2 chromosome, 14 of the 1209 anaphase and telophase figures counted, or 1.1 percent showed these aberrant configurations (B, table 1). They were observed many times in roots where counts were not made.

The fate of the delayed double-sized ring depends upon its position in the spindle figure as the cell-plate appears. If it is in the middle, the cell plate passes through it, dividing it into relatively equal or decidedly unequal segments (figures 19 and 20). If it is not medially placed, the cell-plate passes to one side and the double-sized ring remains in the cytoplasm of one of the daughter cells (figures 21 and 22, and photographs of same, 12 and 16, Plate I). If it lies rather far away from the mid-region, it may be included in one of the nuclei. If this occurs and if normal splitting of this double-sized ring with two spindle fiber attachment regions follows in the next division, two double-sized rings, each with two spindle fiber regions should then be found lying close together in the spindle figure when the two spindle fiber regions on the same chromatid pass to opposite

poles. Several configurations have been observed in which two rings were lying very close together (figures 23 and 24, and photographs of the same, 13 and 14, Plate I). The exact contours of the individual rings could not be accurately followed and therefore have not been shown in the drawings. Since the contours of the two ring chromosomes could not be accurately

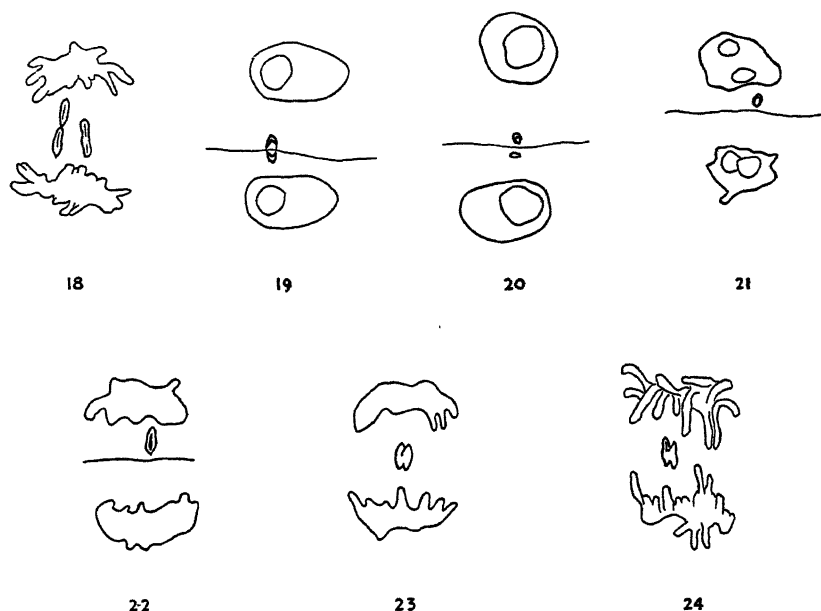


FIGURE 18.—Simultaneous loss at late anaphase of two enlarged R2 chromosomes.

FIGURE 19.—Late telophase appearance after loss of an R2 chromosome. The double-sized ring chromosome has been intercepted by the cell-plate.

FIGURE 20.—Similar to figure 19 but a later stage.

FIGURE 21.—Telophase. The excluded R2 chromosome has not been intercepted by the cell-plate. See photograph 16, Plate I.

FIGURE 22.—Telophase. The excluded enlarged R2 chromosome was not medianly placed in the spindle figure. See photograph of the same, 12, Plate I.

FIGURE 23.—Late anaphase. Two enlarged R2 chromosomes lying close together at the cell-plate region. For description, see text. See photograph of the same, 13, Plate I.

FIGURE 24.—Similar to figure 23. See photograph of the same, 14, Plate I.

followed, such figures could represent two interlocked ring chromosomes from a similar condition to that described above, if instead of a double-sized ring, two interlocked sister ring chromosomes had been included in the preceding telophase nucleus.

Since anaphases and telophases with the ring chromosome lying in the middle of the spindle figure are the most frequent types of aberrant configurations, and since these rings are subsequently excluded from the



telophase nuclei, the frequently observed absence of the ring chromosome from branches of the tassel or from groups of cells in an anther can be explained.

In connection with the problem of the mechanism of movement of chromosomes in the spindle it would be of interest to explain why these small double-sized rings do not appear to be under greater tension as their spindle fiber regions pass toward opposite poles. It is possible that movement toward opposite poles is initiated at the spindle fiber region of the chromosome at early anaphase but that continued movement is made possible by other forces exerted on the chromosomes when they have reached a region in the spindle which is some distance away from the equatorial plate. These double-sized ring chromosomes may be too small to reach this region by the pull exerted at the spindle fiber region. The behavior of intermediate sized rings lends support to this assumption since these sometimes remain in the equatorial plate either with or without evidence of tension.

From cytological examination of sporocytes it was known that changes in size of the small ring chromosomes do occur though with relatively low frequency. The R 2 chromosome has been observed to decrease to several chromomeres and also to increase to seven or eight times its original size. Photograph 15, Plate I, represents a normal R 2 chromosome, photographs 11 and 12, an enlarged R 2 chromosome. It has also been seen that an increase in the number of these rings in a nucleus, usually with alterations in size, sometimes occurs. As many as six ring chromosomes in the nuclei of a sector of a plant which very probably possessed but one ring in the zygote have been observed in a single instance.

If a double-sized ring is included in one of the daughter nuclei, as described above, a start in the direction of increase in number of rings has been made. The chance of loss of this ring chromosome in subsequent divisions is great. However, as already shown, the ring chromosome may be directly broken in two by opposed poleward forces at anaphase, or the poles of the spindle may lie so close together that the spindle fiber regions of the ring are readily included in the two nuclei, after which the resulting chromatin bridges are cut through by the cell-plate and drawn into the nuclei. When two double-sized sister ring chromosomes each with two spindle fiber regions are present in an anaphase figure, and when each of these is subsequently broken and the broken ends drawn into the telophase nuclei, the initial event in the production of a sector of tissue with two altered ring chromosomes has occurred. It is apparent, on this basis, why increase in number of these small ring chromosomes is relatively rare: one infrequent event must be followed by another.

*Conclusions regarding ring chromosome behavior*

The foregoing account has indicated the probable method by which rings are altered in size and genic constitution or are lost from the nuclei altogether. From both cytological and genetical observations it has been concluded that the rate at which this occurs is dependent upon the length of the chromonema composing the ring: the longer the chromonema, the more frequent the occurrence of aberrant mitoses involving the ring. Counts of the aberrant ring chromosome configurations in late anaphase and very early telophase are given in table 1. The counts are from roots in which the ring chromosome was present in most of the cells. In A, the R1 chromosome of case I, figure 1, is represented. One aberrant configuration of the ring chromosome was observed in these roots which were counted. Judging from the relatively small amount of *bm 1* tissue shown by plants with this R1 chromosome, loss of the ring chromosome must be relatively infrequent. In B, the small R2 chromosome of case II is represented. The observed aberrant configurations of the ring chromosome in these roots amounted to 1.1 percent of all the figures recorded. As stated above, these aberrant configurations result mainly in loss of the ring chromosome from both nuclei. Variegation, expressed by the *bm 1* tissue in these plants, is considerably greater than in the plants from which the counts of table 1, A, were obtained. Table 1, C, represents the counts from a plant which possessed two ring chromosomes, a normal R2 and an R2 enlarged approximately three times. In the roots from which the counts were made, both rings were present in many of the nuclei. Aberrant configurations involving the enlarged R2 chromosome were more frequent than those involving the normal R2 chromosome. The size of the enlarged R2 chromosome lies at the border line between those rings whose aberrant configurations lead mainly to exclusion from the telophase nuclei (small rings) and those whose aberrant configurations lead mainly to changes in size of the ring chromosomes (large ring chromosomes). For the enlarged R2, both types of configurations were frequently encountered. In table 1, D, from a plant with a ring chromosome approximately twice the size of the enlarged R2 chromosome, the aberrant configurations amounted to 8 percent of the total number recorded. In this case, as described above, the figure is possibly too low. The telophase figures here were characterized mainly by changes in size of the ring chromosome rather than loss from the nuclei.

If a ring chromosome in a given individual carried a dominant gene and the two normal rod chromosomes carried a recessive gene, the expression of variegation produced by losses or changes in constitution of the ring chromosome would depend upon (1) the size of the ring chromosome and

(2) the position of the gene with respect to the spindle fiber attachment region. In relatively small ring chromosomes, which are mainly lost from the nuclei, the expression of variegation is directly dependent upon the actual size of the ring chromosome: the larger the ring chromosome the greater the amount of variegation exhibited. This is strikingly illustrated by the two ring chromosomes, R 1 and R 2. The variegation produced by R 1 is very much less than that produced by R 2. To check this conclusion without prejudice, cultures were obtained in which either R 1 or R 2 or both R 1 and R 2 chromosomes were expected to be present in individual plants

TABLE 2

*Comparisons of the predicted and observed ring chromosome constitutions in cultures segregating plants with one, two and three ring chromosomes.*

| PREDICTION:<br>1R 1 |            | PREDICTION:<br>1R 2 |           | PREDICTION:<br>2 rings |                            | PREDICTION:<br>3 rings |           |
|---------------------|------------|---------------------|-----------|------------------------|----------------------------|------------------------|-----------|
| Correct             | Deviation  | Correct             | Deviation | Correct                | Deviation                  | Correct                | Deviation |
| 14                  | 1(R 1+R 2) | 15                  | 3(R 1)    | 24                     | 5 (1 ring)*<br>1(3 rings)† | 3                      | 0         |

\* Three showed one R 1; one showed one R 2 in an estimated two R 2 plant; one showed one R 2 in an estimated R 1 plus R 2 plant. Complete agreement in all cases could not be expected in two- and three-ring plants from sporocyte examinations since loss of one of the ring chromosomes in the developmental stages of the tassel is expected in some cases. This particularly applies to the R 2 chromosome.

† The estimate for this plant was two R 1. Some of the two R 1 plants have practically no *bml* tissue. A two R 1 plant could be difficult to distinguish from a three R 2 plant.

of the culture. In some of these cultures plants with three ring chromosomes were expected. From the expression of the variegation exhibited by each plant a prediction was made as to the ring chromosome constitution of the plant. Cytological observations were subsequently made to determine the correctness of these predictions. Table 2 shows the correlation of these observations with the predictions. Cultures of plants with the R 1 chromosome can readily be separated from cultures whose individuals possess the R 2 chromosome through observations of the variegation alone (see following section for more complete discussion).

With relatively large ring chromosomes, which are characterized mainly by changes in size of the ring chromosomes, the expression of variegation would depend upon the nearness of the locus of the gene to the spindle fiber region. The farther away the locus, the greater is the amount of variegation that should be expressed.

The method of alteration of the ring chromosomes as suggested by the somatic anaphase and telophase configurations should produce rod-shaped chromosomes. Although thousands of microsporocytes have been examined in many of which an alteration of the ring chromosome has been ob-

served, no rod-fragments have been recognized. It can not be stated that they do not occasionally occur, but certainly their frequency must be exceedingly low. If the method by which ring chromosomes change in size has been correctly interpreted from the study of somatic anaphase and telophase figures, one is forced to conclude that the broken ends of the chromosomes unite, thus reestablishing a ring.

It might be stated here that when two ring chromosomes are present in the nuclei of a plant, it is rare that both rings show aberrant configurations in the same cell (figure 18). Each ring chromosome apparently acts independently with regard to the formation of double-sized or interlocked rings.

That the behavior of ring chromosomes in maize is a consequence of their form and not of their genic constitution can be definitely stated, since a number of different ring chromosomes, each involving segments of chromosomes not strictly comparable, have been found so far. These include segments from chromosomes II, V, VI, VIII and IX. Most of them were detected by the variegation which they produced but three were isolated independently of any visible effect.

### III. THE NATURE OF THE *Bm 1-bm 1* VARIATION

The gene *bm 1* when homo- or hemizygous produces a brown color of the cell walls. The color appears in the walls as soon as lignification sets in. It is not present before this period. The depth of color, on external examination of *bm 1* plants, is greatest in those tissues which are composed largely of thickened cell walls, such as the midrib of the leaf, the veins in the leaf sheath and the stalk tissue. The brown color is not easily detected in the leaf tissues other than the midrib since the cell walls are thin and the color is masked by the chlorophyll.

As the plant matures in the field, the brown color has been noted to fade considerably in exposed regions of the plant but remains deep in those regions which are well protected from light. It was suspected that direct sunlight was causing a change in the structure of the brown pigment which resulted in loss of color. To determine if this was correct, black paper was placed about exposed parts of several *bm 1* plants when the brown color was intense. The bands of black paper remained about these parts for a period of three weeks. When the paper was removed, the tissues protected from light had retained their original deep brown; the brown color in the tissues above and below the protected region had faded considerably.

In plants possessing two normal chromosomes V with *bm 1* (or one normal chromosome V with *bm 1* and one of the deficient chromosomes V) and a ring chromosome with *Bm 1*, streaks of *bm 1* tissues are pro-

duced and can be seen by external examination of the plants (figure 1). Over 7000 variegated plants have been examined in the progeny of the two original variegated plants. Cytological observations have indicated that loss of the ring chromosome carrying *Bm 1* is the primary cause for the appearance of the *bm 1* tissues. Losses can occur anywhere in the ontogeny of the plant. The patterns of the *bm 1* tissues should give some indication of where and when these losses occurred. Although wide or narrow bands on the stalk (figure 1) indicate an early or late loss of the ring chromosome, respectively, cross sections of the stem, where most of the cell walls are heavily lignified, give even a better indication of the time of loss. If loss occurred early in ontogeny, the whole plant would be *bm 1*. If the first loss occurred in one of the cells which is to give rise to the part of the plant above the ground, a wide sector of *bm 1* would be produced. Still later losses would produce sectors of various widths in the stem. Very late losses would produce streaks or patches composed of a few cells only. All of these types of variegation patterns have been observed.

When a stalk with a relatively wide external band of *bm 1* tissue is cross-sectioned and examined with low magnification, the brown-walled tissue is seen to be composed of a V-shaped sector with the tip of the V pointing toward the center of the stalk. Many narrow surface streaks are produced by similar sectors but the V is smaller and the tip considerably removed from the center of the stalk. Very narrow streaks may be composed of only a few cells. Such streaks are visible on external examination of the stalk only if they lie at or close to the surface. Patches of *bm 1* cells not close to the surface cannot be seen by external examination.

Dilution of color in the brown (*bm 1*) cell walls on the side of the wall adjacent to the white (*Bm 1*) cell walls was a striking feature of the variegation in all plants. That it is a dilution produced by the adjacent *Bm 1* cells and not a spreading of the brown color from the *bm 1* cell walls is suggested by the considerable reduction in intensity of color in the brown walls of the very small patches composed of only a few cells, and by the dilution of color of a row of *bm 1* epidermal cells on the side adjacent to inner *Bm 1* cells.

The variegation in plants possessing an R 2 chromosome is expressed by a few totally *bm 1* plants where the ring chromosome has been lost before the cells which are to produce the stem meristem have been differentiated, to plants which are composed of many *bm 1* streaks of different widths. Cross sections of the stems of the average variegated plant show wide V-shaped sectors, smaller V-shaped sectors and many irregular patches of *bm 1* composed of few to many cells.

The variegation patterns in plants with the R 1 chromosome were similar

to those produced by plants with the R2 chromosome but the total amount of *bm 1* tissue was very much less. There were fewer sectors of all types in these plants, making cultures of the two types of variegated plants readily distinguishable. This is expected from the cytological examinations since the smaller ring chromosome is lost less frequently in somatic divisions than the larger ring chromosome. The extent of variegation is a direct expression of the rate of loss of the ring chromosome.

Plants with two ring chromosomes show considerably less variegation than plants with one ring chromosome. Loss of one ring chromosome followed later by loss of the second ring chromosome or simultaneous losses of both ring chromosomes must occur before the *bm 1* tissue could be produced. The patterns of the *bm 1* tissues in cross-sections of the stem clearly show this relationship. These fall into three main types of sectors: (1) solid V-shaped sectors, (2) spotted V-shaped sectors, and (3) small patches of *bm 1* tissue.

The solid V-shaped sectors are interpreted as relatively early losses of one ring followed slightly later by loss of the second ring chromosome or by occasional simultaneous losses of both rings. The spotted V-shaped sectors reveal more closely the relationship between loss of one ring followed considerably later by losses of the second ring. They are detected as a cluster of *bm 1* patches in an isolated region of a stem which otherwise shows very few *bm 1* patches. When each of the brown patches in such a cluster is traced with a camera lucida and lines drawn joining the outer boundaries of the outermost patches, the lines converge in the direction of the center of the stem. They clearly define a V-shaped sector. Such spotted V-shaped sectors would be expected if loss of one ring carrying *Bm 1* is followed later in development by losses in different cells of the the second ring chromosome carrying *Bm 1*.

The small patches of brown walled tissue, usually composed of only a few cells, can be interpreted as relatively late, successive, or occasionally simultaneous, losses of the two rings.

There are three types of plants with two ring chromosomes: (1) those with two R1, (2) those with one R1 and one R2 chromosome, and (3) those with two R2 chromosomes. Since somatic loss of the R2 chromosome is considerably more frequent than the R1 chromosome, the amount of *bm 1* tissue produced in each of these plants is progressively greater. Plants with two R2 chromosomes have considerable amounts of *bm 1* tissue; those with one R1 plus one R2, very much less, and those with two R1 chromosomes exceedingly little *bm 1* tissue. In this latter type of plant it is often necessary to examine cross-sections of the stem to determine if any *bm 1* tissue is present. Such tissue, when not close to the surface, cannot be detected from field examinations of the plants.

Plants with three ring chromosomes of the constitution two R 2 plus one R 1 chromosome or one R 2 plus two R 1 chromosomes, have been obtained. These plants frequently show no external evidence of *bm 1* tissues. In all cases, however, careful examinations of the stalks have revealed small patches of *bm 1* cells. The *bm 1* cells could arise only after loss (mainly successive) of all three ring chromosomes from the nuclei.

For the sake of comparison, the stalks of a number of plants with a normal chromosome constitution carrying *Bm 1* in one chromosome V and *bm 1* in its homologue were examined. In no case was there any evidence of *bm 1* tissue.

In conclusion it can be emphasized that the genetic expression of variegation is in full agreement with expectation on the basis of the cytological observations given in the previous section. In these plants with small ring chromosomes whose aberrant mitotic configurations are followed mainly by loss of the ring chromosome from the nucleus, the extent of variegation is a direct indication of the length of the chromonema composing the ring chromosome, the larger the ring chromosome the higher the rate of loss and thus, the greater the amount of exhibited variegation. Loss of the ring chromosome can occur at any stage in the development of the plant, early loss giving rise to a totally *bm 1* plant, later loss to wide sectors of *bm 1* and very late losses to small patches of *bm 1* cells. The patterns exhibited by two and three ring chromosome plants are those expected from the cytological observations where it has been shown that simultaneous loss of the several ring chromosomes from a nucleus is rare. The cause of the aberrant mitotic configuration arises independently in each ring chromosome.

Knowledge gained from a study of variegation in these plants has been utilized in the analysis of tissues of plants mosaic for homozygous deficiencies (section V).

#### IV. TYPES OF FUNCTIONAL GAMETES PRODUCED BY THE TWO ORIGINAL VARIEGATED PLANTS

Each of the two original variegated plants possessed one normal chromosome V with *bm 1*, one deficient chromosome V and a ring-shaped fragment chromosome corresponding in size to the deficiency in the rod chromosome (figure 2). In case II (Def 2, R2) prophase meiotic associations had indicated the homology of the ring chromosome with the region in the rod chromosome which had been deleted (figures 25 and 26; photographs of the same, 17 and 18, Plate II). Most frequently, the ring chromosome did not associate with its homologous section in the normal rod

chromosome but remained separate and collapsed (for meiotic prophase behavior of ring-shaped chromosomes, see McCLINTOCK 1933). In all cells the deficient rod chromosome V and the normal chromosome V were associated. The normal V had to buckle to compensate for the deletion in the deficient V. Figures 27 and 28, and photographs of the same, 19 and 20, Plate II, illustrate this association. In the plant from which figure 27

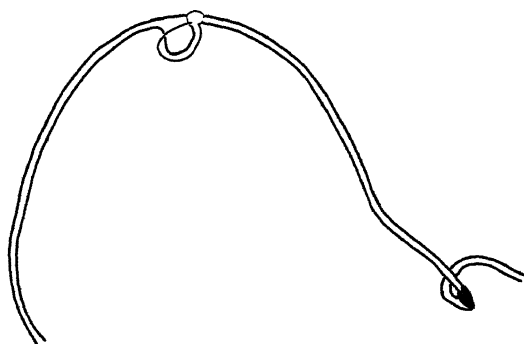


FIGURE 25.—Pachytene association of a normal chromosome V, a Def 2 chromosome V and its compensating R2 chromosome. The ring chromosome has been drawn with a finer line. The spindle fiber attachment region has been drawn as a slight bulge. The dark bodies toward the end to the right are the knobs. See photograph of the same, 17, Plate II.

was drawn, two ring chromosomes were present. They are separate and collapsed. Another figure from the same plant, photograph 21, Plate II, shows the not infrequent association of the two R2 chromosomes to form a true ring-shaped configuration and also the association of the normal and Def 2 chromosomes.

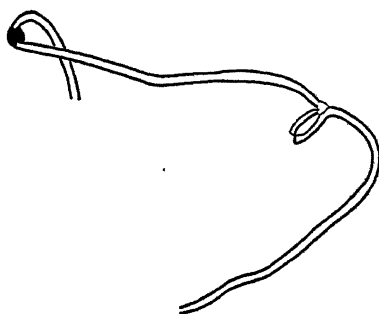


FIGURE 26.—Similar situation to that shown in figure 25. See photograph of the same, 18, Plate II.

In case I (Def 1, R1), no figures were observed showing the association of the ring chromosome with its homologous section in the normal chromosome. It is probable that it occurred in a small percentage of the cases but



would be difficult to detect except in the most favorable figures because of the smallness of the deficiency and the ring chromosome. Figures 29 and 30 illustrate the pachytene association of the Def1 chromosome with

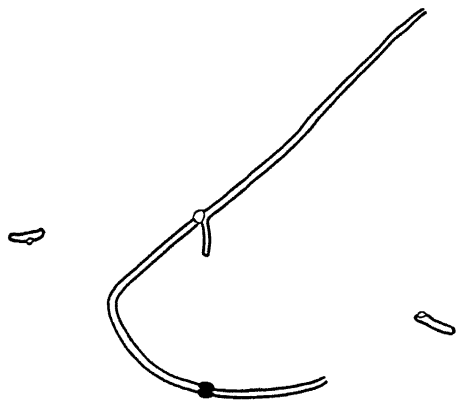


FIGURE 27.—Pachytene association in a microsporocyte of a plant with one normal chromosome V, one Def2 chromosome V and two R2 chromosomes. Note the buckle in the normal chromosome V at the spindle fiber attachment region and the two unassociated, collapsed ring chromosomes. The ring chromosomes have a similar chromatin constitution to that of the buckle. See photograph of the same, 19, Plate II.

a normal chromosome V. The small ring chromosome (R1) lies free and is collapsed. In figure 30 and photograph of the same, 28, Plate II, non-homologous associations about the deficient region have resulted in a

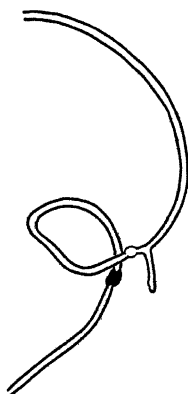


FIGURE 28.—Pachytene association of a normal chromosome V and a Def2 chromosome V. See photograph of the same, 20, Plate II.

separation of the spindle fiber attachment regions of the two chromosomes (for expected non-homologous associations, see McCLINTOCK 1933). The small buckle below the lower spindle fiber region or the distance between

the two spindle fiber regions represents the extent of the deficiency. Photographs 23, 26 and 27, Plate II, illustrate the appearance of the R1 ring at meiotic prophase. In photograph 23, very early diplotene, the

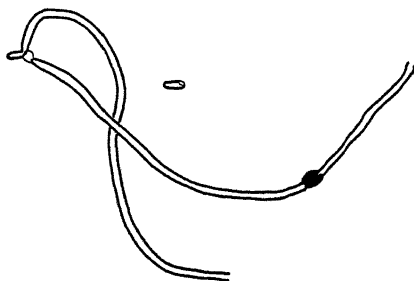


FIGURE 29.—Pachytene association in a microsporocyte of a plant with one normal chromosome V, one Def 1 chromosome V and an R1 chromosome. The ring chromosome is collapsed and is not associated with its homologous region (buckle) in the normal chromosome V.

ring shape of the chromosome is clear. In photograph 26 the spindle fiber region of the R1 chromosome is stuck to that of chromosome VIII. In photograph 27, the spindle fiber region of the collapsed R1 chromosome is adjacent to that of a normal chromosome V bivalent. Photographs 24

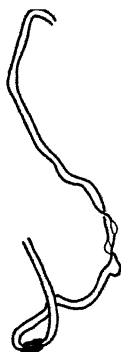


FIGURE 30.—Pachytene association of a normal chromosome V and a Def 1 chromosome V. Through non-homologous associations, the buckle which compensates for the deficiency, has shifted into the long arm of the normal chromosome V. Note the displacement of the spindle fiber attachment regions. See photograph of same, 28, Plate II.

and 25, Plate II, illustrate the relative sizes of R1 and R2 when both are present in the same sporocyte. In both photographs the collapsed ring chromosomes are associated by their spindle fiber attachment regions. In photograph 24 the R1 chromosome is above, the R2, below. In photograph 25 the R1 chromosome is to the left, the R2 to the right.

In plants heterozygous for either deficiency and its compensating ring

chromosome, the deficient rod chromosome and its normal homologue proceed quite normally during the meiotic mitoses, two spores of a quartet receiving the deficient chromosome, two the normal chromosome. The behavior of the ring chromosome, on the other hand is irregular. In the case of R1 chromosome the split halves separate and pass to opposite poles at anaphase I along with the disjoining bivalents (except where double-sized or interlocked ring chromosomes are formed). The split halves of the R2 chromosome likewise separate at I, either at the same time that the bivalents disjoin or slightly later. They are nearly always included in the first division telophase nuclei.

In the second meiotic mitosis, the behavior of the ring chromosome is variable. They do not divide again but either pass to one of the poles along with the other chromosomes of the complement or remain in the spindle figure and are excluded from the telophase nuclei. The behavior of the rings in the two sister cells is not always the same.

As a result of meiosis, four types of spores are to be expected. They carry the following chromosomes:

1. Normal chromosome V.
2. Deficient chromosome V.
3. Normal chromosome V plus the ring fragment.
4. Deficient chromosome V plus the ring fragment.

The percentage of each type in an anther would depend upon (1) the proportion of the sporocytes which lacked a ring chromosome, giving only types 1 and 2 above, and (2) the percentage of cases in which the ring chromosome, when present, was included in the second meiotic telophase nucleus.

Examination of the pollen has given some indication of the percentage of each of these four types which are present in an anther. Pollen from a *bm1* sector of a plant known to have a normal chromosome V (carrying *bm1*), a deficient chromosome V (Def1), and an R1 chromosome, showed three types of grains: (1) large well filled grains, (2) small partially filled grains and (3) small totally empty grains. The proportions of each type are shown in table 3, A. In these *bm1* sectors it is assumed on good evidence (see sections I and II) that the ring chromosome has been lost. Equal proportions of type 1 and type 2 grains should be present. If the small partially filled grains represent those with the Def1 chromosome, the large filled grains, those with the normal chromosome V, they should be present in equal proportions. A total of 5535 normal pollen grains to 5547 small partially filled grains clearly indicates this association. The 347 small empty grains represent 3.3 percent of the total. In all samples of

pollen from normal maize plants there is a small percentage of these empty grains. They probably represent the products of abnormalities in meiosis which are not infrequently observed in normal plants.

Anthers from *Bm1* regions of the plant, in which the ring chromosome is present, give a higher proportion of normal well-filled grains (table 3, B). The difference is interpreted as due to the presence of the ring chromosome in some of the grains which have a deficient rod chromosome (type 4, above). Since the ring chromosome, if unaltered, covers the deficiency, a normal appearing pollen grain is expected. On this interpretation, the number of each type in a particular anther can be estimated. Pollen types 1 and 2 should be present in equal numbers. Likewise, types 3 and 4 should be present in equal numbers. Type 2 grains can be directly recorded. An equal number of the normal appearing grains should belong to type 1. When this number is subtracted from the total number of normal appearing grains, the remainder can be equally distributed to types 3 and 4. The estimates of each type of grain from the *Bm1* anthers in table 3 B, are: type 1, 1994; type 2, 1994; type 3, 743; type 4, 743, or 36.5 percent each of types 1 and 2 and 13.5 percent each of types 3 and 4.

In one plant, heterozygous for Def1 R1, four types of pollen grains were present, 619 large well-filled grains, 170 small but well-filled grains, 426 small partially-filled grains (type 2) and 49 small empty grains. If it is assumed that the small well-filled grains represent type 4 with an altered ring chromosome which does not completely cover the deficiency, pollen types 2 and 4 can be directly recorded. If, on the other hand, these grains are included in the normal appearing class, and calculations made as above, the proportion of types are: type 1, 426; type 2, 426; type 3, 184; type 4, 184. It is obvious that there is a close agreement in the calculated number of 184 for type 4 grains and the 170 grains which have been assumed to represent this type.

In plants heterozygous for Def2 and R2, the type 2 pollen grains are large but almost completely empty. These grains cannot be distinguished from the few empty grains produced by other causes than the presence of the deficiency in chromosome V. However, if these latter grains are assumed to represent two percent of all the grains, an approximate estimate can be made of the number of grains with each of the four chromosomal constitutions. The counts from the *bm1* anthers are given in table 3 C, and similar counts from the *Bm1* anthers, with estimates of proportions of types, in table 3 D.

The functional capacity of each of the four types of gametes can best be illustrated by reference to the types and numbers of individuals resulting from the crosses given in table 4. Section A in the table represents the

TABLE 3

*A. Pollen counts from bm1 anthers of plants with the constitution Def 1/bm1/R1.*

| PLANT   | LARGE FILLED<br>GRAINS | SMALL PARTIALLY<br>FILLED GRAINS | EMPTY<br>GRAINS | % EMPTY<br>GRAINS |
|---------|------------------------|----------------------------------|-----------------|-------------------|
| 598A-2  | 1181                   | 1148                             | 49              | 2.0               |
| 598A-2  | 832                    | 917                              | 38              | 2.1               |
| 597A-2  | 775                    | 751                              | 66              | 4.1               |
| 597A-2  | 610                    | 611                              | 21              | 1.6               |
| 598A-16 | 692                    | 678                              | 42              | 2.9               |
| 598A-3  | 523                    | 526                              | 70              | 6.2               |
| 597B-6  | 922                    | 916                              | 61              | 3.2               |
| Totals  | 5535                   | 5547                             | 347             | 3.3               |

*B. Pollen counts from Bm1 anthers of a plant with the constitution Def1/bm1/R1.*

| PLANT  | LARGE<br>FILLED<br>GRAINS | SMALL<br>PARTIALLY<br>FILLED<br>GRAINS | EMPTY<br>GRAINS | % EMPTY<br>GRAINS | ESTIMATES OF FOUR TYPES<br>OF GRAINS IN PERCENT |    |    |    |
|--------|---------------------------|--|-----------------|-------------------|---|----|----|----|
|        |                           |  |                 |                   | 1   | 2  | 3  | 4  |
| 598A-2 | 1431                      | 802                                    | 47              | 2.0               | 36  | 36 | 14 | 14 |
| 598A-2 | 1094                      | 561                                    | 26              | 1.5               | 34  | 34 | 16 | 16 |
| 598A-2 | 956                       | 631                                    | 28              | 1.7               | 40  | 40 | 10 | 10 |
| Totals | 3481                      | 1994                                   | 101             | 1.8               |   |    |    |    |

*C. Pollen counts from bm1 anthers of a plant with the constitution Def2/bm1/R2.*

| PLANT  | NORMAL GRAINS | EMPTY GRAINS |
|--------|---------------|--------------|
| 953B-6 | 681           | 733          |
| 953B-6 | 943           | 918          |
| 953B-6 | 864           | 962          |
| 953B-6 | 761           | 779          |
| 953B-6 | 1036          | 1079         |
| Totals | 4285          | 4471         |

*D. Pollen counts from Bm1 anthers of plants with the constitution Def2/bm1/R2.*

| PLANT   | NORMAL GRAINS | EMPTY GRAINS | ESTIMATE OF FOUR TYPES OF<br>GRAINS IN PERCENT |    |    |    |
|---------|---------------|--------------|--|----|----|----|
|         |               |              | 1  | 2  | 3  | 4  |
| 1009-10 | 906           | 864          | 48   | 48 | 2  | 2  |
| 1009-8  | 955           | 623          | 38   | 38 | 12 | 12 |
| 1009-8  | 1281          | 847          | 38   | 38 | 12 | 12 |
| 1009-8  | 1109          | 822          | 41   | 41 | 9  | 9  |
| Totals  | 4251          | 3156         |  |    |    |    |

progeny from crosses of the two original variegated plants. Section B represents crosses of plants from A of this table, which were heterozygous for the deficiency and compensating ring, with normal *bm 1* plants. It can be seen that all four gametes produced by plants heterozygous for Def1 R1 can be transmitted through the eggs. Through the pollen, gametes of type 1 and 3 are readily transmitted but gamete type 2 is not transmitted and gamete type 4 only rarely in competition with gametes 1 and 3.

TABLE 4

| TYPE OF CROSS<br>(Female parent to left) | CONSTITUTION OF PLANTS RESULTING FROM CROSSES |                  |                    |                    |
|--|---|------------------|--------------------|--------------------|
|  | <i>bm 1</i> PLANTS                            |                  | VARIEGATED PLANTS  |                    |
|  | <i>bm 1/bm 1</i>                              | Def/ <i>bm 1</i> | <i>bm 1/bm 1/R</i> | Def/ <i>bm 1/R</i> |
| A. Def1/ <i>bm 1</i> /R1× <i>bm 1</i>    | 218   | 9                | 16                 | 12                 |
| <i>bm 1</i> ×Def2/ <i>bm 1</i> /R2       | 38  | 0                | 0                  | 8                  |
| Def2/ <i>bm 1</i> /R2× <i>bm 1</i>       | 352   | 1*               | 13                 | 11                 |
| reciprocal                               | 696   | 4*               | 38                 | 154                |
| B. Def1/ <i>bm 1</i> /R1× <i>bm 1</i>    | 300   | 13               | 29                 | 17                 |
| reciprocal                               | 74  | 0                | 17                 | 1                  |
| C. Def1/ <i>bm 1</i> × <i>bm 1</i>       | 155   | 6                | 0                  | 0                  |

\* These *bm 1* plants probably arose through very early loss of the ring chromosome. The Def2 chromosome is not transmitted without the R2 chromosome.

In plants heterozygous for Def2 R2, gametes 1, 3 and 4 are transmitted through the eggs but gamete 2 is not transmitted. Through the pollen, gametes 1 and 4 are readily transmitted. The frequent transmission of the Def2 R2 combination through the pollen contrast with the very infrequent transmission of the Def1 R1 combination through the pollen. Both cases are in agreement in the lack of transmission of the deficient chromosome minus its compensating ring chromosome through the pollen.

To obtain high counts on transmission of the deficiency carrying gametes, the gene *bt* (brittle endosperm) was introduced into the normal chromosome. The *bt* gene is located in the long arm of chromosome V (RHOADES 1936) and gives very little crossing over with *bm 1*. No positive case of crossing over between *bt* and the two deficiencies of chromosome V has been found. Thus, plants with a deficient chromosome carrying *Bt* and a normal chromosome with *bt*, when crossed to normal *bt* should give the percentage of deficiency-carrying gametes (*Bt* kernels) directly, without the necessity of growing large progenies and testing each indi-

vidual plant for the presence of the deficient chromosome. The results of such crosses are given in table 5. In many of the crosses *bm 1* was likewise involved. The deficiency carrying plants were *Def Bt/Bm 1 bt* with or without a ring chromosome carrying *Bm 1*. These were crossed with normal *bm 1 bt* individuals. The *Bt* kernels should give rise to variegated (*Bm 1-bm 1*) plants when the ring chromosome is present or totally *bm 1* plants when the ring chromosome is absent; the *bt* kernels should give rise to totally *Bm 1* plants. The results of a test of the *Bt* and *bt* kernels are summarized in table 6. If no crossing over occurred between the deficiency and *Bt*, *bm 1* could appear in these crosses only when a deficient chromosome was present. 177 of the 358 individuals showing *bm 1* were tested for the presence of the deficient chromosome. It was present in every case. Thus, the data in table 5 can be used as a direct means of determining the functioning of the gametes which carry a deficient chromosome.

TABLE 5  
*Bt* kernel test of transmissions of *Def 1* and *Def 2* chromosomes.

| CROSS*                                | <i>Bt</i> | <i>bt</i> |
|---------------------------------------|-----------|-----------|
| 1. <i>+Bt/+bt × bt</i>                | 2893      | 2746      |
| 2. reciprocal                         | 797       | 889       |
| 3. <i>Def 1 Bt/+bt/R1 × bt</i>        | 1217      | 6055      |
| 4. reciprocal                         | 4         | 5544      |
| 5. <i>Def 1 Bt/+bt (no ring) × bt</i> | 927       | 12682     |
| 6. reciprocal                         | 0         | 323       |
| 7. <i>Def 2 Bt/+bt/R2 × bt</i>        | 187       | 5735      |
| 8. reciprocal                         | 363       | 1951      |
| 9. <i>Def 2 Bt/+bt (no ring) × bt</i> | 0         | 1319      |
| 10. reciprocal                        | 0         | 4065      |

\* A non-deficient chromosome V is represented by +. The pollen parent is placed at the right in each cross.

The results given in lines 3 and 5 of table 5 are particularly interesting with respect to the functioning of the *Def 1* chromosome through the eggs. If there had been no megaspore selection in favor of the spore carrying the normal chromosome and if all the eggs (or zygotes) which carried the *Def 1* chromosome functioned, the ratio of *Bt* to *bt* should be equal. In all cases the number of *bt* kernels was greater than *Bt* and in all cases the ears were incompletely filled. The presence of the abortive grains on the ear indicate that there has been little if any selection of normal chromosome carrying megaspores. Therefore, the percentage of *Bt* kernels,

when the *bt* kernels are taken as the standard of expectancy, indicates the extent of functioning of the deficiency carrying eggs (or zygotes). The 927 *Bt* kernels in line 5, table 5, represent 7.2 percent of the deficiency carrying eggs (or zygotes) which functioned. In the 74 ears which contributed to this count, the percentages ranged from 0 to 23.2 with half of the ears falling within the range of 2 to 8 percent. When the ring chromosome (R1) was present (line 3, table 5) the *Bt* kernels on the 30 ears contributing to this count ranged from 1 to 40 percent of expectancy on the individual ears and averaged 20.1 percent. That this increase can be attributed to the presence of the ring chromosome covering the deficiency in many of the eggs can be seen from line 1, table 6.

TABLE 6

| CROSS                                    | PLANTS FROM <i>Bt</i> KERNELS |            | PLANTS FROM <i>bt</i> KERNELS |            |
|--|-------------------------------|------------|-------------------------------|------------|
|  | <i>Bm1-bm1</i><br>variegated  | <i>bm1</i> | <i>Bm1</i>                    | <i>bm1</i> |
| Def1 <i>Bt/Bm1 bt/R1</i> × <i>bm1 bt</i> | 40                            | 23         | not grown                     |            |
| Def1 <i>Bt/Bm1 bt</i> × <i>bm1 bt</i>    | 0                             | 85         | 328                           | 0          |
| Def2 <i>Bt/Bm1 bt/R2</i> × <i>bm1 bt</i> | 106                           | 7          | 342                           | 0          |
| <i>bm1 bt</i> × Def2 <i>Bt/Bm1 bt/R2</i> | 95                            | 2          | 24                            | 0          |

That pollen containing the Def1 chromosome without its compensating ring chromosome does not function in competition with normal pollen can be concluded from line 6, table 5. By use of a 170 wire mesh screen these grains, which are small and partially filled with starch, can be segregated from the normal grains and the possible factor of competition with the grains carrying the normal chromosome eliminated. Some of the ears pollinated with sifted pollen gave no kernels at all, others a few *bt* kernels through passage of a few small but normal chromosome pollen grains through the wire mesh. It can be definitely stated, therefore, that these grains are incapable of producing an effective pollination when placed upon normal silks.

Line 4, table 5, suggests that the grains with a Def1 and an R1 chromosome normally do not effect a pollination in competition with normal chromosome carrying grains. The four *Bt* kernels were grown to determine the chromosome constitution of the resulting individuals. Two of these *Bt* kernels were definitely produced through contamination, one through functioning of a Def1 R1 grain and one could have been a cross-over between *Bt* and the deficiency although contamination could not be excluded definitely. If this kernel represents a crossover, it is the only evidence so far obtained of crossing over between the deficiency and *bt*.



From this evidence it could be concluded that (1) the ring-shaped chromosome, R1, does not completely cover the deficiency due to a loss of a small section either at the time of irradiation or during the development of the original plant or that (2) a mutation affecting pollen tube growth appeared at the time of irradiation or (3) the particular chromosomal modification (position effect) is responsible for the reduced pollen tube activity. If (1) above is correct, the deficiency, when homozygous, does not produce a visible effect in the tissues of the mature plant (see section V).

That the gametes with Def2 function only when the ring chromosome, R2, is present is evident from tables 5 and 6. The Def2 gametes with a complete R2 chromosome have an equal chance in competition with normal chromosome carrying gametes. The discrepancy in the percentages of *Bt* kernels in the reciprocal crosses, lines 7 and 8, table 5, can be understood when it is realized that the ear arises from a definite sector of tissue which originally may or may not have had the ring chromosome in its nuclei (6 of the 36 ears had no *Bt* kernels) whereas the pollen is shed from all parts of the tassel which is usually a mosaic of sectors with and without the ring chromosome. The percentage "expected" *Bt* kernels on the 36 individual ears in the cross summarized in line 7, table 5, ranged from 0 to 8.9 percent, those on the six ears summarized in line 8, from 4.5 to 21.6 percent.

As stated in section II, changes in size and genic constitution of the ring chromosomes occasionally occur during ontogenesis of a plant. This being so, it could be objected that the two original ring chromosomes could not be kept constant through successive generations. Small duplications within a ring chromosome are not phenotypically detectable. They must be determined through cytological examination. In contrast, the deficiencies within the ring chromosome can be detected through phenotypic appearances of certain plants (see section V) and through pollen transmissions which tend to eliminate gametes with the deficient ring chromosome. However, change in size of the ring chromosome is not frequent and with proper care, it is not difficult to maintain stocks with unaltered ring chromosomes.

#### V. PRODUCTION OF PLANTS MOSAIC FOR HOMOZYGOUS DEFICIENCIES

As shown in the previous section, the progeny of the crosses of the two original variegated plants by normal *bm1* included a number of individuals with chromosomal constitutions similar to the two original plans. Plants heterozygous for Def1 and R1 when crossed by plants heterozygous for Def2 and R2 should produce twelve types of plants, each with a different chromosomal constitution:

- |                       |                         |                            |
|-----------------------|-------------------------|----------------------------|
| 1. <i>bm1/bm1</i>     | 5. <i>Def1/bm1/R2</i>   | 9. <i>Def2/bm1/R1/R2</i>   |
| 2. <i>bm1/bm1/R2</i>  | 6. <i>Def1/Def2/R2</i>  | 10. <i>Def1/bm1/R1</i>     |
| 3. <i>Def2/bm1/R2</i> | 7. <i>bm1/bm1/R1</i>    | 11. <i>Def1/bm1/R1/R2</i>  |
| 4. <i>Def1/bm1</i>    | 8. <i>bm1/bm1/R1/R2</i> | 12. <i>Def1/Def2/R1/R2</i> |

In the cross of heterozygous *Def1 R1* by normal *bm1* plants, a number of individuals with the constitution of plant type 4, above, were obtained. When these, in turn, are crossed by plants heterozygous for *Def2 R2*, the first six types of plants listed above should be produced.

All of the plants except 6 and 12 from the first cross, and all the plants except 6 from the second cross can be distinguished by the presence or absence of variegation, the type of variegation exhibited and the type of pollen shown by each plant. Cytological examinations of a number of these plants were in agreement with the field determinations.

The appearance of plants of type 6 and 12 could not be predicted. In actual experience it proved very simple to identify them. Both types of plants possess two deficient chromosomes, *Def1* and *Def2*. Plant 6 has one ring chromosome, *R2*, plant 12, two ring chromosomes, *R1* and *R2*. These two types of plants will be designated *R2* double-deficient and *R1 R2* double-deficient.

In the *R2* double-deficient plants, the ring chromosome covers both deficiencies. Its loss in somatic nuclear divisions should result in cells homozygous deficient for the extent of the deficiency in the *Def1* chromosome. If these cells were viable and continued to multiply at the same rate as the surrounding heterozygous deficient cells, which are close to normal in growth rate, both wide and narrow sectors of homozygous deficient tissues should be produced through early and late losses, respectively, of the ring chromosome during ontogeny. In the *R1 R2* double-deficient plants, both ring chromosomes cover the homozygous deficient segment in the rod chromosomes. Simultaneous loss of both ring chromosomes or loss of one ring chromosome followed later by loss of the second ring chromosome must occur in order that homozygous deficient tissue can be produced. The total amount of homozygous deficient tissues produced in the *R2* double-deficient plants should be considerably greater than that produced by the *R1 R2* double-deficient plants. If tissues homozygous deficient for the extent of the deficiency in *Def1* were visibly modified, the two types of plants should be readily distinguishable. However, no prediction as to the nature of the homozygous deficient tissue was possible before the appearance of these plants.

In addition to the *bm1* and variegated plants resulting from the cross of *Def1/bm1* × *Def2/bm1 R2*, one plant appeared (table 7) which was not *bm1* and did not show the ordinary *Bm1-bm1* variation. This plant was stunted in growth habit, the leaves and leaf sheaths were uniformly

TABLE 7  
Def 1/*bm* 1 (no ring) × Def 2/*bm* 1/R 2.

| CULTURE | <i>bm</i> 1 AND<br>VARIEGATED | Def 1/Def 2/R 2 |
|---------|-------------------------------|-----------------|
| 692     | 220                           | 1               |
| 693     | 23                            | 0               |
| 36-30   | 97                            | 0               |
| Totals  | 340                           | 1               |

streaked with fine bands of colorless tissue, as shown in figure 32 (compare with figure 31, a normal leaf). In the cross of Def 1/*bm* 1/R 1 × Def 2/*bm* 1/R 2, besides the *bm* 1 and variegated plants, two new types of plants appeared (table 8). One type was similar to the plant just described. The other type was considerably larger, approaching normal in growth habit, but was not a typical *bm* 1 or variegated plant, and presented the same fine streaks of colorless tissues in the leaves and leaf sheaths as the first new type. However, the total amount of such tissue was markedly less and the pattern of this tissue was not uniform, figures 35 and 36.

TABLE 8  
Def 1/*bm* 1/R 1 × Def 2/*bm* 1/R 2.

| CULTURE | <i>bm</i> 1 AND<br>VARIEGATED | Def 1/Def 2/R 2 | Def 1/Def 2/R 1/R 2 |
|---------|-------------------------------|-----------------|---------------------|
| 694     | 40                            | 1               | 1                   |
| 695     | 41                            | 1               | 2                   |
| 35-9    | 126                           | 0               | 0                   |
| 35-10   | 243                           | 1               | 0                   |
| 35-11   | 134                           | 5               | 1                   |
| 36-24   | 172                           | 2               | 2                   |
| Totals  | 856                           | 10              | 6                   |

It was suspected that the two new types of plants represented the two expected types of double deficient, and that the colorless streaks represented the homozygous deficient tissues. Since these streaks of colorless tissue were not wide, as some of them theoretically should be from homologies with the *bm* 1 streaks in normal variegated plants, it was suspected that the cells of the homozygous deficient tissue were unable to multiply at the same rate as the surrounding heterozygous deficient cells. As stated in section II, loss of the R 2 chromosome occurs much more frequently than loss of the R 1 chromosome. If the rate of loss for each of these two chromosomes is uniform throughout development, a uniform distribution of colorless streaks should be present in the R 2 double-deficient plants.

In the case of the R1 R2 double-deficient plants, early loss of the R1 chromosome should give a sector of tissue with the pattern of colorless streaks characteristic of the R2 double-deficient plants, since the chromosome constitution within the sector is the same. If the R2 chromosome were lost early in ontogeny, a sector of tissue with a different pattern of colorless streaks should result. These colorless streaks should be considerably less frequent since the R1 chromosome is lost from the nuclei less frequently. Nevertheless, the distribution of such streaks should be uniform. If this hypothesis were correct, the small, uniformly but heavily streaked plants should be the R2 double-deficient plants, the larger, non-uniformly streaked plants, R1 R2 double-deficient plants. That these two types represented the expected R2 and R1 R2 double-deficient plants was established through cytological observations and confirmed by pollen examinations and appropriate crosses.

In the intercrosses of plants heterozygous for Def2 and R2, the union of a Def2 R2 gamete with a similar gamete results in a plant with two Def2 chromosomes plus two R2 chromosomes. (It is of theoretical interest to point out that the chromosome number has been increased in these plants without changing the genome complement, that is, these 22-chromosome plants are genomically equivalent to normal 20-chromosome plants.) Since these plants are markedly different from the double-deficient plants, a description will be postponed until section VII. The functional gametes produced by these plants contain the Def2 chromosome plus one or two R2 chromosomes. The gamete most frequently transmitted through the pollen contains but one R2 chromosome. In the crosses of Def1/*bm1* and Def1/*bm1*/R1 by plants homozygous for Def2 and R2, all the eggs which carry the normal chromosome with *bm1* should give rise to normal variegated plants, all those that carry a deficient chromosome to double-deficient plants. The results of these two types of crosses are given in tables 9 and 10. The test for the functioning of deficiency-carrying eggs is similar to the *Bt* and *bt* tests described in the previous section. The correlation between the proportions of functional

TABLE 9  
Def1/*bm1* (no ring) × Homozygous Def2, R2.

| CULTURE | NORMAL <i>Bm1</i> — <i>bm1</i><br>VARIEGATED* | Def1/Def2/R2 |
|---------|---|--------------|
| 814     | 76  | 0            |
| 36-28   | 61  | 8            |
| 36-29   | 135   | 20           |
| 37-56   | 13  | 0            |
| 37-57   | 64  | 0            |
| Totals  | 349   | 28           |

\* A few plants were *bm1*, see page 364.

TABLE 10  
Def1/*bm1*/R1 × Homozygous Def2, R2.

| CULTURE | NORMAL <i>Bm1-bm1</i><br>VARIEGATED* | Def1/Def2/R2 | Def1/Def2/R1/R2 |
|---------|--------------------------------------|--------------|-----------------|
| 36-25   | 106                                  | 18           | 15              |
| 36-26   | 56                                   | 9            | 4               |
| 37-52   | 154                                  | 17           | 12              |
| 37-53   | 175                                  | 22           | 31              |
| 37-54   | 107                                  | 2            | 5               |
| 37-55   | 162                                  | 5            | 18              |
| Totals  | 760                                  | 63           | 85              |

\* A few plants were *bm1*. See page 364.

eggs with a normal chromosome V, a deficient chromosome V, and a deficient chromosome V plus its ring chromosome, respectively, is similar in the two tests.

It remains to be shown that the colorless streaks in the double-deficient plants represent the homozygous deficient tissues produced after loss of the ring chromosomes during somatic mitosis. Adequate confirmation of this relationship is obtained from the patterns of such tissues in double-deficient plants with the following ring chromosomes: one R1, one R2, two R2, one R1 plus one R2, two R1 plus one R2, one R1 plus two R2. Double-deficient plants with different combinations of ring chromosomes can be obtained from crosses of R2 and R1 R2 double-deficient plants by plants homozygous for Def2 R2 and from intercrosses of the double-deficient plants. The results of the respective crosses are given in tables 11, 12, and 13. It should be noted that only plants homozygous for Def2 R2 and double-deficient plants result from these crosses.

Classification of these plants into the two categories of homozygous

TABLE 11  
Def1/Def2/R2 × Homozygous Def2, R2.

| CULTURE | HOMOZYGOUS<br>Def2, R2 | Def1/Def2<br>one R2 | Def1/Def2<br>two R2 |
|---------|------------------------|---------------------|---------------------|
| 823     | 0                      | 1                   | 0                   |
| 987     | 0                      | 2                   | 5                   |
| 988     | 2                      | 3                   | 1                   |
| 989     | 9                      | 11                  | 13                  |
| 990     | 4                      | 18                  | 2                   |
| 991     | 7                      | 4                   | 4                   |
| 992     | 3                      | 20                  | 6                   |
| 37-85   | 1                      | 7                   | 1                   |
| 37-86   | 3                      | 17                  | 5                   |
| 37-87   | 0                      | 15                  | 1                   |
| 37-88   | 1                      | 9                   | 1                   |
| 37-89   | 4                      | 20                  | 7                   |
| 37-90   | 4                      | 8                   | 3                   |
| 37-91   | 4                      | 18                  | 2                   |
| Totals  | 42                     | 153                 | 51                  |

TABLE 12

Def 1/Def 2/R 1/R 2  $\times$  Homozygous Def 2, R2.

| CULTURE | HOMOZYGOUS | Def 1/Def 2 | Def 1/Def 2 | Def 1/Def 2      | Def 1/Def 2 |
|---------|------------|-------------|-------------|------------------|-------------|
|         | Def 2, R2* | ONE R 2     | TWO R 2     | ONE R 1, ONE R 2 | THREE RINGS |
| 37-58   | 11         | 30          | 9           | 14               | 3           |
| 37-59   | 6          | 10          | 11          | 16               | 2           |
| 37-60   | 5          | 8           | 2           | 4                | 0           |
| 37-61   | 1          | 0           | 0           | 8                | 0           |
| 37-62   | 1          | 11          | 11          | 0                | 0           |
| 37-63   | 3          | 14          | 3           | 30               | 2           |
| 37-64   | 3          | 14          | 0           | 33               | 4           |
| 37-65   | 5          | 5           | 0           | 4                | 3           |
| 37-66   | 0          | 7           | 0           | 8                | 2           |
| 37-67   | 4          | 11          | 2           | 13               | 8           |
| 37-68   | 0          | 2           | 1           | 6                | 2           |
| 37-69   | 1          | 6           | 1           | 12               | 5           |
| 37-70   | 3          | 1           | 1           | 6                | 5           |
| 37-71   | 2          | 8           | 6           | 14               | 2           |
| 37-72   | 14         | 16          | 3           | 20               | 6           |
| 37-73   | 12         | 16          | 5           | 27               | 17          |
| 37-74   | 5          | 8           | 5           | 19               | 3           |
| 37-75   | 6          | 15          | 1           | 20               | 3           |
| Totals  | 82         | 152         | 61          | 254              | 67          |

\* Some of these plants had, in addition, an R 1 chromosome.

Def 2 R 2 and double-deficient was simple since the former type of plant has a peculiar growth habit (see section VII) and does not show the particular streaks which are always present in the double-deficient plants. The double-deficient plants, in turn, were classified as to their ring chromosome constitution on the basis of the patterns of the colorless streaks. If the colorless streaks represent the homozygous deficient tissue, then the pattern exhibited by the double-deficient plants with the R 1 or R 2 chromosome or various combinations of two or three of these rings should

TABLE 13

Plants obtained from sib crosses of Def 1/Def 2/R 1/R 2.

| CULTURE | HOMOZYGOUS | Def 1/Def 2 | Def 1/Def 2 | Def 1/Def 2 |
|---------|------------|-------------|-------------|-------------|
|         | Def 2, R2* | 1 RING      | 2 RINGS     | 3 RINGS†    |
| 37-76   | 4          | 15          | 15          | 9           |
| 37-77   | 1          | 14          | 27          | 11          |
| 37-78   | 3          | 0           | 19          | 5           |
| 37-79   | 5          | 6           | 20          | 8           |
| 37-80   | 0          | 3           | 4           | 6           |
| 37-81   | 2          | 0           | 9           | 1           |
| 37-82   | 6          | 17          | 21          | 17          |
| 1000    | 0          | 8           | 27          | 11          |
| 1001    | 9          | 1           | 14          | 9           |
| 1003    | 2          | 7           | 22          | 10          |
| Totals  | 32         | 71          | 178         | 87          |

\* Several of these plants had, in addition, an R 1 chromosome.

† Several of these plants were suspected to have four rings.

be predictable from the knowledge of their behavior in somatic mitosis (section II) and from the knowledge gained from a study of the patterns of *bm1* tissues in normal variegated plants with these same combinations of ring chromosomes (section III). A number of these plants were examined cytologically to establish the value of the prediction. The results are summarized in table 14. The agreement between predicted and observed is obvious from the table.

TABLE 14

*Comparisons of predicted and observed chromosomal constitutions.*

| PREDICTED CONSTITUTION<br>FROM APPEARANCE OF PLANT | NO. PLANTS<br>EXAMINED | DEVIATION FROM<br>EXPECTATION |
|--|------------------------|-------------------------------|
| Homozygous Def2, R2                                | 17                     | 0                             |
| Def1/Def2+one R2                                   | 18                     | 0                             |
| Def1/Def2+two R2                                   | 10                     | 1 (1 R1)                      |
| Def1/Def2+one R1 and one R2                        | 24                     | 1 (1 R1)                      |
| Def1/Def2+two R1 and one R2                        | 5                      | 2 (2 R2; 1 R1+2 R2)           |
| Def1/Def2+one R1 and two R2                        | 6                      | 1 (R1+R2)                     |
| Totals   | 80                     | 5*                            |

\* See footnote \*, table 2.

The patterns of colorless tissue exhibited by the various double-deficient plants will be briefly described. Photographs of parts of leaves of double-deficient plants with various ring chromosome combinations are given in figures 31 to 38. To conserve space only a small part of a leaf is shown, which considerably limits the effectiveness of the demonstration.

The colorless streaks in the one R2 double-deficient plants are uniformly distributed throughout the leaf area and are relatively closely spaced (figure 32). Double-deficient plants with the R1 chromosome have been produced only by very early loss of the R2 chromosome in plants originally possessing both the R1 and R2 ring chromosomes. There is considerably less streaking but the distribution of these streaks is uniform (figure 34). Plants with two R2 chromosomes are clearly distinguishable from those with an R1 and R2 chromosome. In both types of plants the streaking is not uniformly distributed. The two R2 chromosome plants have a considerably greater total amount of colorless tissue. There are numerous sectors of various widths with a pattern similar to that shown by the one R2 plants (figure 33, sector to right). This is to be expected since loss of either ring chromosome would give rise to cells with the same chromosome constitution as the one R2 plants. Sectors with the R1 pattern, figure 34, are not found. The R1 R2 double-deficient plants have fewer streaks than the two R2 plants. The sectors in these plants are either of the one R2 type (figure 36, sector to left), or of the R1 type (figure 35, sector to right of mid-rib), through early loss of the R1 or R2 chromosome, respec-

tively. In the three ring double-deficient plants, very few colorless streaks were observed. This is particularly evident in the two R1 plus one R2 plants. Many of the leaves in these plants have no well defined sectors but only scattered streaks here and there. Sectors, when present, are

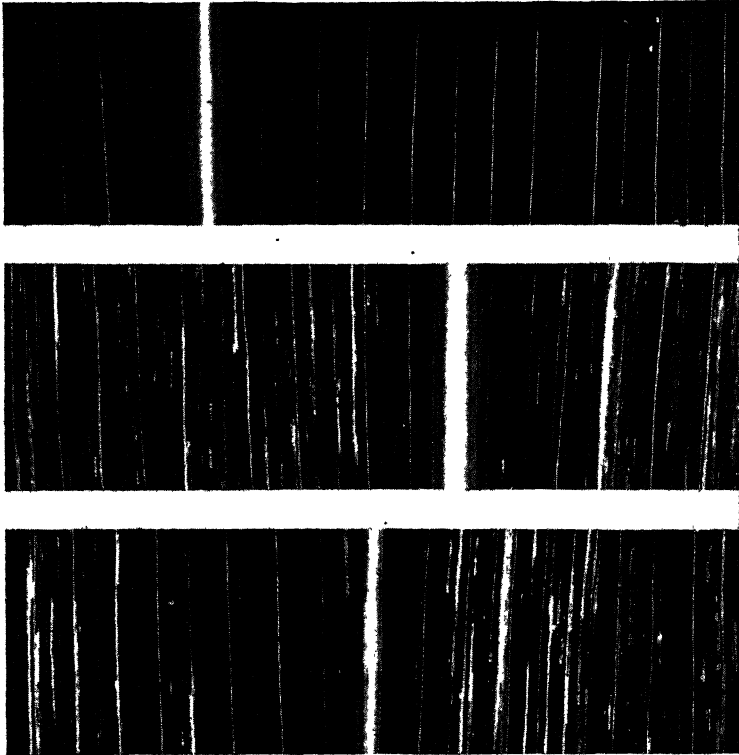


FIGURE 31.—(upper) Surface view of a small region of a leaf of a plant with a normal chromosome constitution. The wide clear band is the midrib. The finer parallel bands are the veins.

FIGURE 32.—(middle) Surface view of a small region of a leaf of a plant with Def1, Def2 and an R2 chromosome. The wide clear band is the midrib. Note the many fine streaks of colorless homozygous deficient tissue.

FIGURE 33.—(lower) Surface view of a small region of a leaf of a plant with Def1, Def2 and two R2 chromosomes. Note the sector to the right of the midrib composed of many fine streaks (one ring sector) and the sector immediately to the left of the midrib with comparatively few streaks (two-ring sector).

usually narrow (figures 37, 38). The two R2 plus one R1 plants have more streaking and more well defined sectors.

That the colorless streaks represent the homozygous deficient tissues resulting from loss of the ring chromosome from the nuclei seems certain from the correlations of the patterns of this tissue in the six types of plants.

The amount of homozygous tissue in a double-deficient plant bears an



inverse relation to the size of the plant, the greater the total amount of homozygous deficient tissue present, the smaller the plant. The one R2 double-deficient plants are smaller than the two R2 plants, which in turn, are smaller than the R1 R2 plants. The three ring plants are practically equal in size and vigor to plants with a normal chromosome constitution.

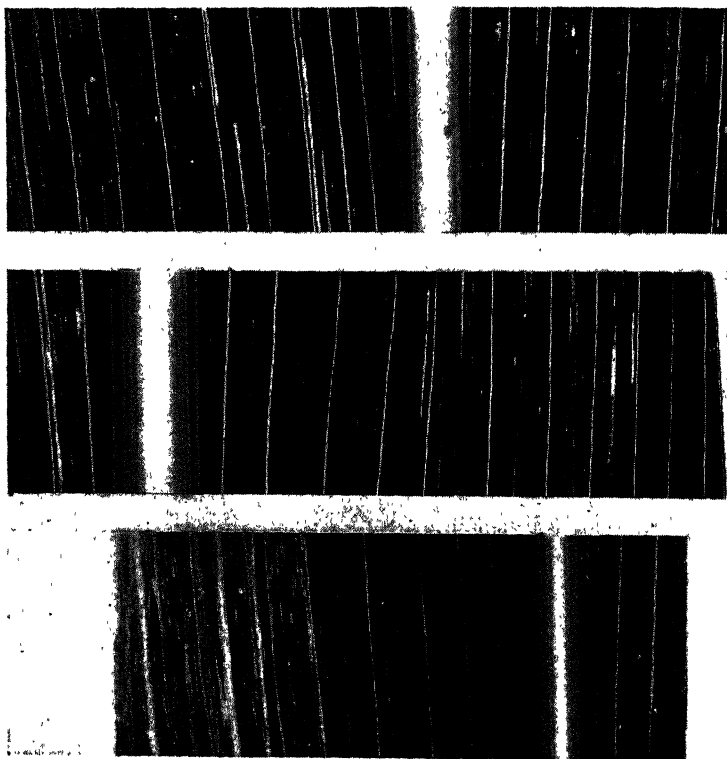


FIGURE 34.—(upper) Surface view of a small region of a leaf of a plant with Def1, Def2 and one R1 chromosome. Note the distribution of streak of homozygous deficient tissue. Compare with figures 32 and 33.

FIGURE 35.—(middle) Surface view of a small region of a leaf of a plant with Def1, Def2 and R1 and an R2 chromosome. Note the R1 sector in the middle of the region to the right of the midrib and the sectors to either side of it which are comparatively free of streaks of homozygous deficient tissue.

FIGURE 36.—(lower) Surface view of a leaf of a plant with the same constitution as that in figure 35. Note the R2 sector (left) and the comparatively small number of homozygous deficient tissue streaks in the tissue to the right.

From homologies of the *bm1* sectors in normal variegated plants (section III), wide sectors of homozygous deficient tissues in the double-deficient plants would be expected to be found if the cells of such tissues could grow and multiply at the same rate as the surrounding cells. It is

reasonable to assume that these cells, with a deficient chromosome complement, would be incapable of an equal growth rate. The juxtaposition of two tissues with unequal growth rates should cause considerable distortion of the cells about the boundaries of the two tissues. This is obvious from the microscopic observations of the two types of tissues in the double-deficient plants. The normal tissues appear to be pulling in the direction of the homozygous deficient tissues. The more rapid growth of the normal

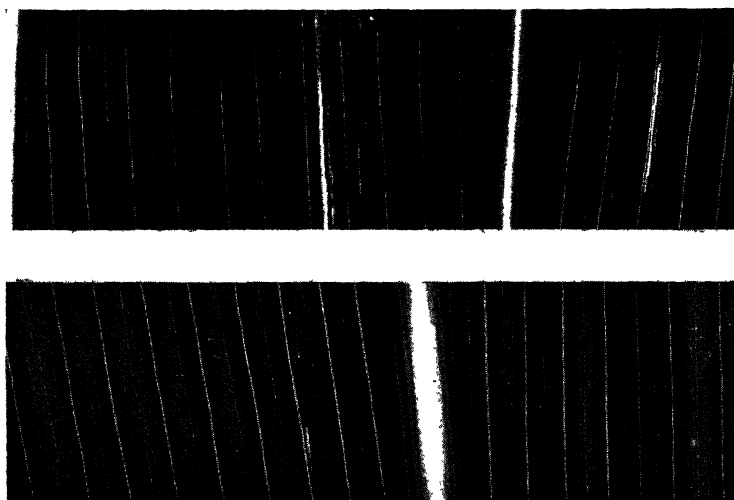


FIGURE 37.—(upper) Surface view of a small region of a leaf of a plant with Def1, Def2, two R2 and one R1 chromosomes. Note the two narrow sectors with streaks of homozygous deficient tissue.

FIGURE 38.—(lower) Surface view of a small region of a leaf of a plant with Def1, Def2, two R1 and one R2 chromosomes. Note the narrow sector to the left of the midrib with a few streaks of homozygous deficient tissue.

tissues may exert enough pull upon the homozygous deficient cells to cause separation at cell boundaries and the production of a hole in the midst of a patch of homozygous deficient tissue. In the one R2 double-deficient plants, which have the most homozygous deficient tissue, the unequal growth rates of the two types of tissue is reflected in a roughened, finely corrugated surface of the considerably reduced and narrowed leaf.

The evidence for considering the colorless streaks as tissues homozygous deficient for the extent of the deficiency in the Def1 chromosome can be briefly summarized:

1. Plants which show these colorless streaks must contain two deficient rod chromosomes, one of which must be the Def1 chromosome. This has been proven by cytological examination, pollen examination and appropriate crosses. Plants homozygous for Def2 R2 do not show the colorless streaks characteristic of the double-deficient plants. It will be shown in

section VII that cells homozygous for the full deficiency in the Def2 chromosome (which is more than twice as long as that in the Def1 chromosome) are incapable of surviving.

2. Plants with these colorless streaks can arise only in crosses where double-deficient plants are expected. For confirmation, see tables 7 to 13.

3. When streaked plants are crossed to normal plants, no streaked plants should appear in the progeny. This has been fully confirmed.

4. The amount of streaked tissue present in a plant and the pattern exhibited should be correlated with the number and kinds of ring chromosomes present. (See table 14 and previous discussion.)

5. A reduced growth rate in the cells homozygous deficient for such a *relatively* large section of the chromosome is to be expected. This is reflected in the small size of the colorless streaks and the distortion of tissues produced by the juxtaposition of tissues with unequal growth rates.

In mentioning the crosses given in tables 11, 12 and 13, little was stated concerning the functional gametes produced by the double-deficient plants. The one R2 double-deficient plants produce four types of gametes (1) Def1, (2) Def2, (3) Def1 R2, (4) Def2 R2. As shown previously, the gamete with Def2 (2 above) will not function in pollen or ovule; (1) above will function in some but not all of the eggs in which it is present but will not function through the pollen. Gamete (4) is equally viable through the eggs and pollen (tables 11 to 13). Gamete (3) is a new type the functioning of which had to be tested. It was found to function readily through the eggs. That it does not function through the pollen in an appreciable amount in competition with (4) is shown by the following test. Pollen of R2 double-deficient plants was placed upon silks of normal *bml* plants. The resulting plants should be variegated (*Bm1* and *bml*) and heterozygous for either Def1 or Def2. Since both types of plants can be distinguished through pollen examinations (see section IV), the pollen of 171 plants resulting from this cross was examined. All showed the presence of the Def2 chromosome. Cytological verification was obtained from 28 of these plants. From this evidence, it can be concluded that type (4) pollen grain is the only normally functioning grain produced by these plants.

The functional gametes of the R1 R2 double-deficient plants have been determined. Of the eight possible gametes: (1) Def1, (2) Def1 R1, (3) Def1 R2, (4) Def1 R1 R2, (5) Def2, (6) Def2 R1, (7) Def2 R2, (8) Def2 R1 R2, the egg can transmit all except (5) and possibly (6). The pollen transmits only (7) and (8).

The dissimilarity in functional capacity of the different types of gametes in pollen and ovules is reflected in the *Pr* and *pr* ratios (*Pr*, purple aleurone; *pr*, red aleurone) in reciprocal crosses (table 15). *Pr* is located in the long arm of chromosome V, 18-24 units from *Bm1* (EMERSON, BEADLE and

FRASER, 1935; the percentage of crossing over varies within this range in different strains). As is obvious from the discussion in this paper, *Bm 1* is very close to the spindle fiber region in the short arm of chromosome V. A measure of the crossing over between the deficiency (or spindle fiber region) and *Pr* can be obtained directly from the crosses shown in table

TABLE 15  
*Dissimilarities in reciprocal crosses.*

| CROSS  | <i>Pr</i>   | %            | <i>pr</i>   | %            |
|--|-------------|--------------|-------------|--------------|
| Def 1 <i>pr</i> /Def 2 <i>Pr</i> /R2× <i>pr</i><br>reciprocal    | 60<br>5192  | 31.5<br>81.3 | 130<br>1198 | 68.5<br>18.7 |
| Def 1 <i>Pr</i> /Def 2 <i>pr</i> /R2× <i>pr</i><br>reciprocal    | 85<br>495   | 61.2<br>19.0 | 54<br>2106  | 38.8<br>81.0 |
| Def 1 <i>pr</i> /Def 2 <i>Pr</i> /R1/R2× <i>pr</i><br>reciprocal | 177<br>4964 | 27.8<br>83.7 | 458<br>973  | 72.2<br>16.3 |
| Def 1 <i>Pr</i> /Def 2 <i>pr</i> /R1/R2× <i>pr</i><br>reciprocal | 165<br>313  | 66.6<br>18.0 | 83<br>1422  | 33.4<br>82.0 |

16. Since no ring chromosome is present, only the pollen grains carrying the normal chromosome function. Crossing over is not altered in plants heterozygous for Def1 and very little in plants heterozygous for Def2.

TABLE 16

| CROSS                                   | <i>Pr</i> | <i>pr</i> | % CROSSING OVER |
|---|-----------|-----------|-----------------|
| <i>pr</i> ×Def 1 <i>Pr</i> /+ <i>pr</i> | 2789      | 9175      | 23.3            |
| <i>pr</i> ×Def 1 <i>pr</i> /+ <i>Pr</i> | 1286      | 425       | 24.8            |
| <i>pr</i> ×Def 2 <i>Pr</i> /+ <i>pr</i> | 562       | 2201      | 20.3            |
| <i>pr</i> ×Def 2 <i>pr</i> /+ <i>Pr</i> | 823       | 184       | 18.2            |

In double-deficient plants, with one Def1 and one Def2 chromosome, crossing over is similar to that in plants heterozygous for the Def2 chromosome, "reciprocal" crosses (table 15).

#### VI. SIMULATION OF THE *bm 1* PHENOTYPE THROUGH LOSS OF THE *Bm 1* LOCUS

When one-ring and two-ring double-deficient plants were closely examined, fine streaks of brown tissue were seen in the leaf sheath, the midrib and the veins of the leaf. These fine streaks were many times more frequent in the one-ring plants than in the two-ring plants. The shade of color and its association with cells having thickened walls were strikingly similar to the effect produced by brown midrib (*bm 1*). In some of the streaks it was obvious that the brown color of the vein was associated

with adjacent parenchyma cells lacking chlorophyll. Since it was known that normal *bm 1* produces a brown color in the lignified cell walls, which can be seen in sections of any lignified tissue, the leaf sheaths with brown streaks were removed, sectioned fresh and examined microscopically. It was immediately observed that the brown color was in the cell walls. It was similar in its range of color and in its deposition in the cell wall to that of ordinary *bm 1*. When brown patches appeared in regions where plastids are normally lacking, that is, heavy-walled schlerenchyma cells, the adjacent parenchyma cells, when too thin-walled to show the brown clearly, frequently lacked plastids. When the adjacent plastid deficient parenchyma cells possessed thickened walls, a brown color could be seen in these walls. The brown-walled and plastid deficient cells formed a definite unit of tissue. It was suspected that these small sectors represented homozygous deficient tissues and that a brown pigmented cell wall would accompany all such tissues. However, the walls of the cells in the colorless streaks in the leaf, except about the veins, are too thin, and the concentration of brown pigment insufficient for a visible effect. If the homozygous deficient cells, produced by loss of the ring chromosome from the nuclei, have brown cell walls, cross-sections of the stem of the single-ring double-deficient plants should show many small, uniformly distributed patches of cells with brown cell walls, just as the leaf is uniformly streaked with fine stripes of colorless tissue. This proved to be true for each of the many R2 double-deficient plants examined. The sections of the stem were advantageous in relating the brown-walled cells to those which lacked plastids since the normally plastid-carrying parenchyma in the outer region of the stem is thick walled and has a sufficient concentration of brown to be readily seen. Considerable distortion of the bundles and cells was present, particularly in the regions about relatively large patches of brown-walled cells. The types of distortion suggested that the brown-walled cells had grown at a slower rate than the surrounding white-walled cells.

In contrast to the single-ring plants, cross-sections of stems of the two-ring plants showed fewer brown patches. Such would be expected if the brown color were limited to cells which were homozygous deficient; loss of one ring followed by loss of the second ring must occur before the homozygous deficient cells could be produced. Frequently a cluster of brown patches was present in a restricted region of the stem. When the extent and position of these brown patches were traced with a camera lucida and a boundary drawn about the cluster, it was clear that they formed one continuous sector. In the two R2 double-deficient plants, the number and distribution of the brown patches in such a sector were similar to those of the one R2 plants. In the R1 R2 plants, some of such sectors were similar to the above and some had considerably fewer patches in a cluster.

In the former plant, the sectorial clusters of brown patches correspond to the one-ring sectors seen in the leaf through early loss of one of the R 2 chromosomes. In the latter plant, the two types of sectorials correspond to early losses of the R 1 or R 2 chromosome, respectively.

If the brown-walled, plastid-deficient patches of cells represent the homozygous deficient tissue, the three-ring double-deficient plants should show very few such patches. When present, their distribution should correspond to that observed in the leaves of these plants. The results obtained were in complete agreement. There were very few such patches in these three-ring plants. This is especially true of the two R 1 plus one R 2 plants.

It might be stated here that plants homozygous for Def 2 R 2 do not show these patches of brown-walled, plastid-deficient cells. The homozygous deficient cells in these plants, as will be shown in the next section, are inviable.

(1) The correlation of the brown cell walls with the cells lacking plastids, (2) their presence in narrow streaks, (3) the restricted growth capacity of these cells, (4) their uniform distribution in one-ring double-deficient plants, (5) their reduced frequency in two R 2, R 1 R 2, and three-ring plants, respectively, and (6) the distribution patterns in sectorials in these two- and three-ring plants summarize the homologies of the brown-walled cells with the streaks of colorless cells in the leaf which were shown to be homozygous deficient in the previous section.

The brown-walled cells are homozygous deficient for the full extent of the deficiency in Def 1. At this point it should be emphasized that the locus of *Bm 1* is carried by the ring chromosome and that there is no locus for *Bm 1* or *bm 1* in the deficient rod chromosomes. If the brown color in the walls of these homozygous deficient cells is similar to *bm 1*, it should appear in the development of the wall at the time lignification sets in, as has been shown for normal *bm 1* (section III). Furthermore, the brown color of the walls in such cells, when adjacent to white-walled cells, should be diluted on the side adjacent to the white-walled cells, as observed in normal variegated plants. Thirdly, when exposed to intense light, the brown color should fade just as normal *bm 1* fades on exposure to light.

Immature cells are present at the base of each leaf sheath. These cells rapidly merge into fully mature cells just above this region. If a leaf showing a prominent brown streak is removed and serial sections made to trace the brown streak as it emerges from the immature cells, it becomes obvious that the brown color appears as the cell walls become lignified in a manner fully comparable to normal *bm 1*. Thus, the time of appearance of the color in the development of the wall is similar to that in normal *bm 1* plants.

When examining the brown patches in sections of the stem it was ob-

served that the color of the brown in the walls of the plastid deficient cells was considerably diluted on the side adjacent to the white-walled, plastid containing cells. This, in turn, is comparable to the observations in normal variegated plants.

To test the third correlation, fading of the color when exposed to light, black paper was placed over part of a conspicuous brown streak in a leaf sheath or midrib of a leaf. Upon removal, several weeks later, the brown color under the paper had retained its intensity, that above and below had lost much of its intensity. In this respect, the brown of the homozygous deficient tissues is similar to normal *bm 1*.

The range in color of normal *bm 1* varies in some plants from a deep wine red to a light orange, the deep red color being present in the stem toward the basal nodes, the light orange in the regions toward the top of the plant. This same gradation of color in comparable regions was found in the brown patches of the double-deficient plants.

To summarize: The two browns, the normal *bm 1* and the brown produced in cells possessing no locus for this gene, are comparable in (1) time of appearance of the color in the development of the cell walls, (2) in dilution of the color in regions adjacent to white-walled (*Bm 1*) cells, (3) in loss of intensity of color when exposed to light, and (4) in range of color variations in specific regions of the plant. No differences could be detected in the expression and behavior of the brown color in the two cases. Although it has not been proven that *bm 1* is due to a deficiency in chromosome V, it can be stated that absence of the locus of *Bm 1* will duplicate the phenotypic expression of *bm 1*.

#### VII. THE PRODUCTION AND APPEARANCE OF PLANTS HOMOZYGOUS FOR Def2 R2

Mention of plants homozygous for Def2 R2 has been made in the previous section. The first of these plants appeared in the progeny of sib crosses of Def2/*bm 1*/R2 through the union of two gametes each containing Def2 and R2. Such plants were to be expected. However, as in the case of the double-deficient plants, no prediction could be made as to appearance other than that they should not be typical *bm 1* or variegated plants. Nevertheless, they are readily recognizable. They are short, usually deep green in color, with thickened, erect leaves and do not show normal *Bm 1*-*bm 1* variegation. The streaks of colorless tissue, so characteristic of double-deficient plants are absent from the leaves.

In later generations many plants homozygous for Def2 R2 were obtained. Cytological examination of microsporocytes at pachytene has confirmed the accuracy of the phenotypic classification (table 14). The two deficient chromosomes V synapse homologously throughout their length.

The changed arm ratio produced by the deficiency is clearly evident. In the normal chromosome V, the chromomeres adjacent to the spindle fiber region on the short arm are relatively large and deep-staining. With the removal of this section in the production of the ring chromosome, small, light-staining chromomeres are brought adjacent to the spindle fiber region, making this deficient chromosome V readily recognizable at pachytene. The two ring chromosomes either synapse to form a ring-shaped bivalent, similar to the rings in photographs 21, 22, Plate II, or remain unsynapsed and appear as two collapsed rings, similar to the rings in photograph, 19, Plate II. There is no tendency for the ring chromosomes to synapse with any part of the deficient rod chromosomes V.

The pollen of these plants is always highly abortive. Only those grains possessing a ring chromosome are normal in appearance and capable of functioning.

The loss of one ring chromosome followed by loss of the second ring chromosome should give rise to cells homozygous deficient for the full extent of the deficiency in the rod chromosomes. If these cells were viable and capable of multiplying, evidence of such tissue would be expected in the leaves of these plants from homologies with the double-deficient plants described in the previous two sections. Since evidence of such tissue did not appear in the leaf, it was suspected that the cells whose nuclei were homozygous deficient for this relatively long section were inviable or incapable of further multiplication. Longitudinal sections of growing points of roots of these plants were made with the view of finding evidence of the fate of these cells since such cells necessarily are formed.

In all root meristems of plants homozygous for Def2 R2, the following peculiar cell type was found. It was confined to roots of these plants, not being present in normal or double-deficient plants. Very much enlarged, heavily vacuolate groups of two or more cells in positions indicating relation in descent, were observed in regions of the root where enlarged cells are not normally encountered (figures 39, 40, 41). When a mitotic figure was observed in one of these cells, the chromosomes were short, thickened and sometimes irregularly placed in the spindle. Daughter telophase nuclei were sometimes joined by a connecting nuclear bridge. In older regions of the root, degeneration processes in these cells, depicted first by an aberrant staining reaction of the nucleolus followed by a pycnotic condition of the cytoplasm (figure 42), and finally by a collapse of these cells due to the pressure of the normal surrounding cells (figure 43). Should the space formerly occupied by these cells be extensive, the surrounding cells divide in planes other than normal, filling in this space which might otherwise have remained as a hole in the tissue (figure 43). The numbers of such patches of enlarged cells varied considerably in different roots. Some had many, others relatively few.



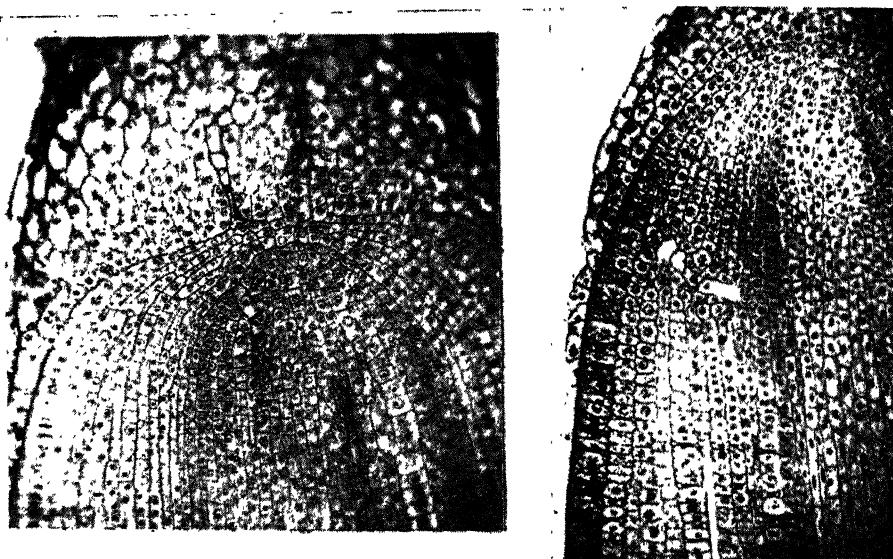


FIGURE 39.—(left) Longitudinal section of a root tip of a plant homozygous for Def2 and R2. Note the two adjacent, enlarged (homozygous deficient) cells near the tip of the growing point and the row of enlarged, degenerating (homozygous deficient) cells in the root cap directly above. Mag.  $\times 160$ .

FIGURE 40.—(right) Similar to figure 39. Note the transverse row of four enlarged (homozygous deficient) cells. Mag.  $\times 160$ .

The conditions depicted conform strictly to expectancy if these cells represent the homozygous deficient cells resulting from loss of the ring chromosomes from their nuclei. Loss of one ring chromosome would cause no obvious tissue alteration since tissues heterozygous for Def2 are normal in appearance. Loss of the second ring chromosome, such loss taking place at anaphase by being left at the equatorial plate of a mitotic figure, would result in two adjacent cells whose nuclei would be homozygous deficient for the extent of the deficiency in the Def2 rod chromosomes. The occurrence of pairs of enlarged cells has been mentioned. In many cases, it was possible to see the cast-out ring chromosome in the cytoplasm of one of these cells but also in many cases, degeneration processes in the cytoplasm had advanced too far for such a determination. No ring chromosome was observed in the few cells which had mitotic figures but the small ring chromosome could have been obscured by one of the rod chromosomes so that evidence of the homozygous deficient conditions of these cells from direct observations of the chromosome constitution was not considered satisfactory.

The numbers of such patches of cells and their distribution in the different roots lend strong supporting evidence for the homozygous deficient



FIGURE 41.—Longitudinal section of a root tip of a plant homozygous for Def2 and R2. Note the row of very much enlarged (homozygous deficient) cells. Mag.  $\times 160$ .

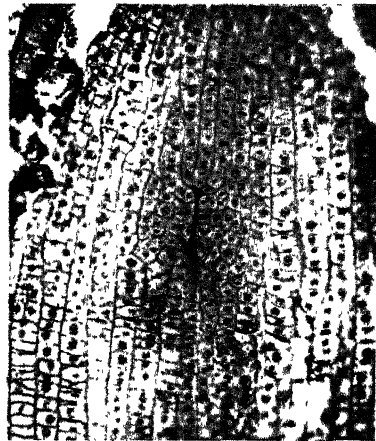
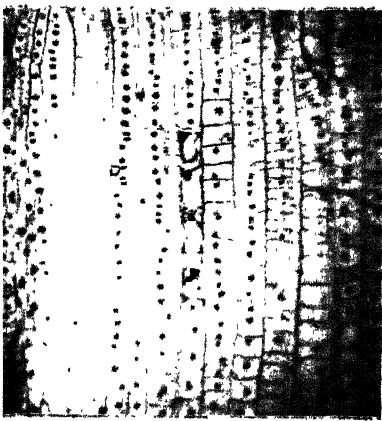


FIGURE 42.—(left) Longitudinal section immediately below the actively meristematic region of a root of a plant homozygous for Def2 and R2. Note the row of four enlarged, degenerating (homozygous deficient) cells. Mag.  $\times 160$ .

FIGURE 43.—(right) Longitudinal section of a root tip of a plant homozygous for Def2 and R2. Note that there has been a proliferation of cells about the degenerated streak. Mag.  $\times 160$ .

nature of these cells. They were present in all roots but the frequency was variable. In a root in which only one ring chromosome is present in the nuclei of the normal cells, a high frequency of such patches should be

observed. In this latter case, every loss of the ring chromosome would be reflected in a patch composed of two or more such cells. In roots where two ring chromosomes are present in many of the nuclei, loss of one ring chromosome followed by loss of the second ring chromosome or the very occasional simultaneous loss of both ring chromosomes would have to occur to produce such a patch. Thus, the frequency of such patches in these roots would be considerably less than in the former type of root. Since a root could contain but one ring chromosome in its normal cells or be a mosaic of one- and two-ring sectors of various sizes, variations in the numbers of patches of abnormal, enlarged (homozygous deficient) cells in the different roots of the homozygous Def2 R2 plants is to be expected.

(1) The presence of these patches of abnormal cells only in plants homozygous for Def2 R2 and their absence in double-deficient and normal plants, (2) the observed presence of the R2 ring in the cytoplasm of one of these cells in many cases, (3) the frequency and distribution of these patches in different roots, (4) their arrangement in descent, that is, rows of two or more, (5) the rapid disintegration of these cells, and finally (6) the absence of evidence of homozygous deficient tissues in the mature cells of the stalk and leaves of these plants as contrasted with double-deficient plants, strongly support the view that they represent the homozygous deficient cells since such cells *must* be produced in these plants. Since early death is the fate of these cells, it is clear why the leaves of these plants are not streaked with modified tissues which could be interpreted as representing the homozygous deficient tissues.

At this point it might be mentioned that a third deficiency (Def3), not previously considered in this paper, which is outside the limits of Def1 but within the limits of Def2 and therefore covered by the R2 chromosome, produces the same pattern of homozygous deficient tissues as that exhibited by the R2 double-deficient plants when the constitution of the plant is Def2/Def3/R2. However, in this case, the homozygous deficient tissue has an even poorer growth capacity than tissues homozygous for the deficiency in the Def1 chromosome. To return to the Def2 chromosome, the deficiency is apparently too long, the loci deleted too important in cell physiology for survival of cells which are homozygous deficient for this region.

When pollen of plants homozygous for Def2 R2 is placed upon silks of normal *bm 1* plants, the progeny should all be variegated for *Bm 1* and *bm 1* except in those cases where the ring chromosome has been lost sufficiently early in development to be absent from that part of the embryonic tissue which produces the visible part of the plant.<sup>2</sup> In this latter case,

<sup>2</sup> Functional male gametes without a ring chromosome, which would give rise to *bm 1* plants, might be produced as the result of a loss of the ring chromosome in the second microspore mitosis.

the plant would be *bm1*. The progeny of 13 such crosses totalled 1,829 variegated to 68 *bm1* plants. To exclude the possibility that this 3.5 percent of *bm1* plants represented contaminations, 42 of them were examined for the presence of the deficient chromosome. In 41 of these plants the deficient chromosome was present; one represented a contamination.

The number of kernels which develop on the ear of a plant homozygous for Def2 R2 is always very small, ranging from 0 to 30 kernels with the average about 10. This would be expected since only those gametes which possess a ring chromosome are functional. Many of the ears should arise from one-ring sectors and others should be composed of both one- and two-ring sectors. Since, in the two-ring sectors, the two small ring chromosomes frequently do not synapse, or if so, do not remain together at the first meiotic mitosis, their elimination in the two meiotic mitoses is frequent. Relatively few ovules with eggs containing a ring chromosome would be expected and thus only a few kernels should be expected on an ear. Since the exertion of anthers and shedding of pollen is dependent upon the presence of a number of well-formed grains in the anther sac, pollen collected from such plants usually contains enough functional grains (those with a ring chromosome) to produce a complete set of seed when placed on silks of normal plants. Since both deficient chromosomes are similar, there is no selection in favor of one or the other chromosome either through the pollen or eggs. Reciprocal backcrosses of *Pr/pr* plants give 1:1 ratios.

#### VIII. PHENOTYPIC EFFECTS OF ALTERED RING CHROMOSOMES

As stated in section II, ring-shaped chromosomes not only are lost from nuclei during somatic mitoses but also change in size. They may increase in size through duplications of segments composing the ring or decrease in size through losses of segments. The frequency of such changes depends upon the size of the ring chromosome. In the case of the small R2 chromosome, such changes are relatively infrequent. Cytological examinations of large numbers of plants and many nuclei within each plant have given abundant evidence, however, of such changes. The R2 chromosome has been seen to increase to approximately seven times its original size and to have decreased to several chromomeres. The duplicated segments do not result in tissues showing extensive modification. Slight modifications are visible with higher multiples resulting in smaller plants with thickened, erect leaves.

In the case of the double-deficient plants, loss of the ring chromosome

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In this case, the tube nucleus would contain the ring chromosome and could be expected to function normally in pollen germination. Thus, sperm nuclei lacking an R2 chromosome, could be introduced into the embryo sac.

results in viable tissues which are homozygous deficient for the extent of Def1, as shown in the previous sections. This tissue is poor in growth capacity, has brown cell walls similar to normal *bm 1*, possesses no plastids and, on continuous exposure to sunlight this tissue in the leaves dries and shrivels. In Def1 the four chromomeres adjacent to the spindle fiber attachment region on the short arm of chromosome V have been removed. The R2 chromosome includes these four chromomeres plus the next five chromomeres.<sup>3</sup> Should changes in size of the ring chromosome delete one or more of the four chromomeres covering the deficiency in Def1 or fractions of them, tissues homozygous deficient for sections within the limits of the deficiency in Def1 should result. Since tissues homozygous for the full extent of the deficiency in Def1 are viable, fractional deficiencies within this region might be expected to have even better viabilities and should reveal themselves by wider sectors of homozygous deficient tissue having a specific modification, this modification should be repeatedly encountered as a sectorial in large populations of such plants. In other words, the nature of these sectorials should vary depending upon the fraction of the four chromomeres which has been deleted from the ring chromosome. The same region should be independently deleted in a number of instances and therefore the same type of sectorial should be produced in a number of different double-deficient plants.

A large number of sectorials have been found. These are classified as simple mutant sectorials, involving a single recognizable change, and compound mutant sectorials, which are combinations of the simple mutant types. Only those sectorials which are readily recognizable because of their good growth capacity are included in this classification. The simple mutant sectorials are as follows: (1) *translucent white* ("onion skin") with colorless cell walls, no plastids; (2) *opaque white*, with colorless cell walls, colorless plastids; (3) *deficiency-brown-midrib*, similar in detail to tissues homozygous for the *bm 1* gene; (4) *pink* colored tissues with colorless cell walls, colorless plastids; (5) *blotched* chlorophyll pattern. The following types of compound mutant sectorials have been found: (1) pink, deficiency-*bm*, viable in sunlight; (2) pink, deficiency-*bm*, dries in sunlight; (3) opaque white, deficiency-*bm*; (4) blotch, dries in sunlight; (5) blotch, deficiency-*bm*, dries in sunlight. The compound mutant sectorials are far more frequent than the simple mutant sectorials. The summation of the characters exhibited by these sectorials is equal to the characters present in tissues homozygous for the full extent of the deficiency in Def1, with the exception that the tissues with the total deficiency are incapable of growing at

<sup>3</sup> This is an estimate of the number of chromomeres. The chromomeres in maize appear to be compound and may show more or less in a particular region depending upon the state of contraction or the degree of stretching in a particular preparation.

a rate which will result in a large sector, whereas the simple mutant sectorials outlined above more nearly approximate the normal growth rate.

The theory is proposed that the simple mutant effects are due to losses of one particular region from the ring chromosome, each mutant effect being related to one particular region. The compound mutant effects are then produced by losses of several adjacent regions within the ring chromosome. On this theory, a linear arrangement of the mutant effects can be referred to the chromosome in the following order: pink, deficiency-*bm*, dries in sunlight and blotch. Translucent white must be removed from deficiency-*bm* and opaque white close to it. The presence of a ring chromosome in the cells of a sectorial has been determined cytologically when the sectorial included a part of the tassel. If an altered ring chromosome was present in the mutant sectorials, streaks of tissue homozygous deficient for the full extent of the deficiency in *Def1* should appear within these sectors through loss of this altered ring chromosome. This is especially easy to confirm in sectorials of deficiency-*bm* and blotch.

In double-deficient plants with two ring chromosomes, the same types of sectorials are encountered. Here, however, all of the sectors are not solid; many of them are variegated. The explanation is similar to variegated patterns of *Bm1* and *bm1* in plants which have two normal chromosomes V carrying *bm1* and two ring chromosomes carrying *Bm1* and to the patterns of homozygous deficient tissues in double-deficient plants with two ring chromosomes. One ring chromosome has suffered a deletion. Production of homozygous deficient tissues showing the character of the deletion can occur only after loss of the second normal ring chromosome. Thus the sectorial is a variegate of normal and mutant tissues. Should the altered ring chromosome be lost in a somatic mitosis many generations later than that which produced the alteration, the tissues resulting after this loss will appear as normal one-ring double-deficient tissue inserted into variegated tissue since only the one normal ring chromosome is left in this tissue. These variegated sectors, mosaics of two types of patterns, are frequently encountered in the two-ring double-deficient plants.

If the theory that the mutant sectorials are due to losses of specific regions within the ring chromosome is correct, plants homozygous for *Def2 R2* should show mutant sectorials similar to those exhibited by the double-deficient plants plus a number of types not exhibited by these latter plants since the extent of the deficiency in *Def2* includes the same four chromomeres plus five more. This has been fully realized. Simple and compound mutants of pink, deficiency-*bm*, blotch, and opaque white plus an additional number of chlorophyll and growth types have been found.

Should a change occur in the ring chromosome, it should remain un-

altered for most of the cell generations and thus should be capable of being passed from one generation to the next, provided the original alteration occurred in tissues which give rise to the gametes. A female gamete containing a deficient rod chromosome plus an altered ring chromosome united with a male gamete with the deficient rod chromosome and a normal ring chromosome would give rise to plants which are complete mosaics of normal and changed tissues. The changed tissues should be of the same type in an individual plant. In some cases there should be many female gametes in the original plant with this same altered ring chromosome if the alteration occurred early in the ontogeny of the ear. Thus there should be many individuals in a culture resulting from the outlined cross each exhibiting the same type of variegated pattern involving the identical mutant characters. This has been realized in one culture. These plants are useful for cytological determinations of the nature of the modification in the ring chromosome but a detailed account of this will appear in a later paper. It is not the purpose of this paper to consider all the evidence concerning changed rings and their mutant effects. It is too extensive. It is necessary to mention it, however, since a comprehension of the mitotic behavior of ring chromosomes would lead one to anticipate the presence of such changed rings with phenotypic effects.

#### DISCUSSION

The presence of ring-shaped chromosomes and a suggestion as to their inconstant behavior in somatic tissues was first published by NAWASHIN (1930; see also, 1936) for a single plant of *Crepis tectorum*. Since this time, ring-shaped chromosomes have been found or produced in *Drosophila* (L. V. MORGAN 1933; STURTEVANT and BEADLE 1936; SIDOROV, SOKOLOV and TROFIMOV 1936; SCHULTZ and CATCHESIDE 1937), *Trillium* (HUSKINS and HUNTER 1935), *Locusta* (WHITE 1935), *Pisum* (ATABEKOWA 1936), *Tradescantia* (HUSTED 1936), *Tulipa* (UPCOTT 1937) and *Nicotiana* (CLAUSEN, unpublished). In none of these cases has an intensive cytological study been made to determine the mitotic behavior of the ring chromosomes. In *Zea* a number of ring-shaped chromosomes have been found (McCLINTOCK 1932, 1933, and unpublished; RHOADES and McCLINTOCK 1935; CREIGHTON, unpublished; CAMERON, unpublished). In all cases studied, the mitotic behavior of these ring chromosomes has been similar. Deletions of sectors from the ring, duplication of sectors, additions in numbers of ring chromosomes of varying constitutions and total loss of the ring chromosome from the nuclei have been observed. In *Drosophila*, phenotypic effects which could be definitely ascribed to alterations in constitution of the ring-shaped X chromosome have not been described. The chances of detecting such an altered ring chromosome would depend

on the rate at which aberrant anaphase figures would be formed. As pointed out in section II, the rate at which alterations occur in ring chromosomes in maize depends upon the length of the chromonema composing the ring. Since the primary cause of double-sized and interlocking rings, the first step in the production of altered ring chromosomes, has not been determined, it is difficult to argue that the behavior found in *Crepis*, *Nicotiana* (R. E. CLAUSEN, unpublished) and *Zea* would likewise be found in *Drosophila*.

HUSTED (1936) reported double-sized, continuous ring-shaped chromosomes, in some of which the two chromonemata made a half turn around each other, and interlocked rings in the microspores of *Tradescantia* following irradiation. Such configurations should be expected if, before irradiation, the chromosomes were split and the two chromatids were relationally coiled about one another. Although the *Tradescantia* cases were not followed beyond the microspore stage, HUSTED attempted to explain the method by which such figures could be produced throughout the life of a plant. The presence of a continuous ring with two spindle fiber attachment regions or two interlocked sister ring chromatids at somatic anaphase, whatever the method by which it originally arose, would result in a chromatin bridge the strands of which would eventually break. HUSTED assumes that each chromosome is split at somatic anaphase. "A broken end of an anaphase chromatid (caused by breakage of continuous or interlocking rings) may unite as often with the broken end of its sister as with its other broken end. Ring chromosomes which are continually breaking might persist in this way. Whenever the two broken ends of one chromatid unite, however, and the two sister strands are *not twisted*, a 'disjunctional' [two sister halves free to disjoin] ring-shaped chromosome would result. There would be a trend toward displacement of the 'continuous' and 'interlocked' types by 'disjunctional' rings which separate without breaking unless relational coiling of chromatids is increased before each union of broken ends" (page 551). The disjunctional rings, once established, should remain free from further complications. Such a theory of the continuous appearance of double-sized and interlocked ring chromosomes throughout the life of a plant cannot account for the origin of such configurations in maize, although it may contribute to some of the cases. This arises from the following consideration. In plants homozygous for *Def2* and *R2*, the two split halves of the ring chromosomes separate freely from one another at anaphase I in most of the sporocytes, that is, are "disjunctional," not continuous or interlocked. Thus, the ring chromosome in the majority of the spores has been derived from a ring chromosome whose two split halves have separated freely in the previous division. As seen in section VII, only those gametes which possess a ring chromosome



are functional. If HUSTED's theory of the continuous appearance of double-sized and interlocked rings were correct for maize, most of the individuals resulting from the cross of normal *bm 1* by homozygous Def2 R2 should be totally *Bm 1* through elimination of the cause of loss of the ring chromosomes in somatic mitoses. As shown on page 364, all plants resulting from the cross are variegated for *Bm 1* and *bm 1*. The cause of the interlocked or double-sized rings arises anew and is dependent upon the length of the chromonema in the ring for its frequency. The primary cause of these configurations may be related to the occurrence of a crossover between the two split halves of a ring chromosome. The high frequency of normally disjoining ring chromosomes, even when the chromonema of the ring is long, leads one to conclude that the plane of splitting or method of reproduction of a new chromonema from an old, is definitely predetermined along a given plane and that trouble might arise only during or after the split had occurred at some point of tension or torsion in the chromosome, that is, a tension relieved by an interchange of segments at this point, resulting in a somatic crossing over between the two chromatids. Unless two crossovers occurred in a chromosome, only continuous, double-sized rings would result. As noted in section II, there was a high frequency of such continuous, double-sized rings as compared to other possible complicated configurations. The maize chromosomes are too small in somatic cells to give a clear picture of the configurations in each cell other than in those with simple continuous rings. On this theory, interlocked rings could arise (1) after two somatic crossovers in which the second crossover did not counteract the first, or (2) following a previous anaphase break and reunion of broken ends in which a twist in the chromonema was present before the union occurred. In this latter case, it is not necessary to assume that the chromosome is split before union of broken ends occurs as interlocked or continuous rings could arise depending upon the plane of splitting or reproduction of the chromonema assumed. On this hypothesis, the proportion of interlocked rings to continuous rings would be expected to be greater with large ring chromosomes than with small ring chromosomes. Likewise, sister nuclei in these plants should show aberrant ring configurations more frequently than sister nuclei in plants with small ring chromosomes.

Since no rod-shaped fragment chromosomes have been observed to arise from ring-shaped chromosomes through breakages in somatic anaphase and telophases, it has been assumed that union of broken ends must occur. In an effort to determine if two broken ends which enter a nucleus would unite, the following experiment was outlined. A plant was made heterozygous for two inversions on two different chromosomes. The inversions did not include the spindle fiber attachment regions and therefore, should give

bridges at anaphase I (or II) and free fragments after a crossover within the inverted segment (McCLINTOCK 1933; MÜNTZING 1934; SMITH 1935; RICHARDSON 1936; DARLINGTON 1936; UPCOTT 1937; SAX 1937 and others). With normal crossing over, the size of the fragment is constant for any particular inversion. In the two inversions chosen, the size of the fragment produced by each was readily distinguishable. In many sporocytes of the plants with the inversions, two chromatin bridges with their respective fragments produced by a crossover in each of the two inversions, were present at anaphase I. In most cases, the bridges of chromatin had broken by late telophase and the broken ends had been drawn into the telophase nuclei. Thus broken ends from two chromosomes entered the same nucleus. If fusion of these broken ends occurred, the product of this fusion should be obvious in some of the second division figures in the cells of which two recognizable fragments were present. In maize, the two second meiotic anaphase figures are oriented in one plane and thus can be observed together. However, the evidence for fusions was negative. It was then considered that the broken ends might be too far apart from one another, in many cases, to join together before the second meiotic mitosis. Therefore, cases of double-crossing over in plants heterozygous for a single inversion were investigated. When a four-strand double crossover occurs within the inverted region, a double bridge involving all four chromatids, and two free fragments of similar size are found in anaphase I. The strands composing these bridges break and the two broken ends, lying side by side, enter the same nucleus. If fusions of these broken ends occurs in each telophase I nucleus, each sister cell in anaphase II should show a chromosome involved in an anaphase bridge. The sister cells to be examined can be distinguished by the two fragments of recognizable dimensions. The evidence for fusions of broken ends entering the same nucleus was likewise mainly negative in this case. On the supposition that each chromatid might already be split in anaphase I and that fusions occurred between broken ends of the two split halves of a chromatid rather than between the broken ends of each chromatid, anaphase configurations in the microspores of these plants were examined. Such fusions should give rise to a chromatin bridge at anaphase of the first mitosis in the spore (see Sax 1937). Upon examination, a chromosome showing a bridge configuration was found in a number of spores. By a method which will be described in a separate publication, it was possible to show that the spores which have a bridge configuration likewise possess a chromosome which was broken during the meiotic mitoses. The examinations indicated, also, that such fusions probably always occur. Such evidence illustrates, directly, the tendency of broken ends to fuse. One would be tempted to use this information and transfer the process to the somatic chromosomes. How-

ever, the evidence at present indicates that one is not justified in doing so. Until the contradictory features of this evidence are completely analysed, the author is unwilling to interpret the ring chromosome behavior on this basis.

Viable homozygous deficiencies in *Drosophila* giving effects similar to "genes" known to be located in the region which has been made deficient, have been described by MULLER (1935) for yellow and achaete, EPHRUSSI (1934), STERN (1935) and DEMEREC and HOOVER (1936) for yellow, STURTEVANT and BEADLE (1936) for scute, EMMENS (1937) for roughest-2, and OLIVER (1937) for facet. Viable individuals homozygous deficient for a region possessing no known genes have been described by DEMEREC and HOOVER. Homozygous deficiencies producing immediate or delayed effects in zygotes and embryos, which eventually result in death to the individual, have been described by POULSON (1937). As far as the author is aware, homozygous deficiencies in plants which simulate a gene known to be located in the deleted segment, have been found only in the *bm1* case described in this paper. Evidence that the known genes in the *Drosophila* cases might be due to deficiencies in the regions involved, has been given only for the gene roughest-2. Simulation of the known gene by a region deficient for its locus has been claimed for the other cases and applies likewise to the *bm1* case in *Zea*. It is unprofitable at present to estimate to what extent homozygous deficiencies are responsible for known genic effects. That some of these may be related to position effects seems possible from the accumulating evidence in *Drosophila*. In how many of these cases the factor of a minute deficiency can be eliminated, remains to be decided, granting that the presence of a deficiency introduces the possibility of a position effect. In the case described in this paper, the two independent segments of chromosome V, the deficient rod and the unaltered ring fragment, produce effects, with regard to the *Bm1* character, which are indistinguishable from that produced by a normal chromosome V carrying *Bm1*. The only evidence so far obtained of a "position effect" is derived from the appearance of plants homozygous for Def2 and R2 and from the lack of expected transmission through the pollen of gametes with Def1 R1 or Def1 R2. In neither case can the factor of a minute deficiency be eliminated, the deficiency affecting plant growth in the former case and pollen transmission in the latter case.

The method of producing phenotypic effects by alterations in ring chromosomes in plants with two deficient chromosomes plus a covering ring fragment, briefly described in section VIII, should prove useful in analysing in considerable detail the genetic composition of small sections of chromosomes. The effects produced have been ascribed to minute homozygous deficiencies rather than to position effects since homozygous

deficiencies must be produced by alterations in the ring chromosomes. If individual regions within the deficient segment produce particular effects independent of their neighbors, combinations of these effects should be produced by loss from the ring chromosome of two or more of these regions. Since the method by which the ring chromosomes become altered should delete adjacent segments from the ring chromosome, an orderly arrangement of compound effects should result. The order of the particular regions within the ring chromosome which have specific effects could be developed from analyses of the individual effects contributing to the compound effects. Since the results obtained so far substantially correspond to the requirements of this theory, the notion of a particulate nature of the composition of this region of the chromosome has been retained. The development of this method of analysing the composition of sections of chromosomes has just been started. It would be premature to draw rigid conclusions from the results so far obtained. Three deficiencies of chromosome V, each of which can be covered by a ring fragment, are available for this study. Two of these fall within the range of the third. Correlations of results from all three deficiencies should conform to a predicted pattern if the above theory is correct. Until the evidence from these studies has accumulated, no attempt will be made to force a particulate theory of the organization of the chromosome in contradistinction to a continuum theory. The former will be retained as a working hypothesis until the evidence definitely requires a modified view.

#### SUMMARY

1. Two cases of a deficiency adjacent to the spindle fiber attachment region in the short arm of chromosome V involving approximately  $1/20$  (Def1) and  $1/7$  (Def2) the length of the chromosome, respectively, were produced by X-ray treatment. The piece deleted in each case formed a small ring-shaped chromosome, R1 and R2, respectively. In each case a section of the spindle fiber attachment region was removed to the ring chromosome and a section was retained by the deficient rod chromosome. Since the deficient rods and compensating ring chromosomes possessed a functional section of the spindle fiber attachment region, each was capable of participating successfully in the mitotic process. The ring chromosome in each case carried the locus of *Bm1* (allele of *bm1*, brown mid-rib, producing a brown color in the lignified cell walls). The rod chromosomes lacked the locus for *Bm1*.

2. Plants with two normal chromosomes V carrying *bm1* (or one normal chromosome V carrying *bm1* and a deficient chromosome V) plus either ring chromosome are variegated for *Bm1* and *bm1* through frequent losses of the ring chromosome from the somatic nuclei.

3. Somatic loss of ring-shaped chromosomes is described.
4. The abnormal mitotic behavior of large and small ring-shaped chromosomes is contrasted. Large ring-shaped chromosomes frequently change in size and chromatin constitution during somatic mitotic cycles. Small ring-shaped chromosomes are more frequently lost from nuclei during mitotic cycles although changes in size and chromatin content sometimes occur.
5. The frequency of aberrant mitotic configurations of the ring chromosomes, leading to loss or change in size, is related to the length of the chromonema composing the ring. The longer the chromonema the more frequent the aberrant configurations. With small ring-shaped chromosomes, whose aberrant mitotic configurations usually lead to loss of the ring chromosome from the nuclei, the extent of variegation (2 above) is proportional to the size of the ring chromosome.
6. Functional gametes with Def1, Def1 plus R1, and Def2 plus R2 were obtained. The functional capacities of these two deficiencies with various combinations of the ring chromosomes were tested. Some of these were functional, others were not.
7. Plants with Def1/Def2/R2 (R2 covers the deficiencies in the rod chromosomes) were a uniform mosaic of tissues heterozygous and homozygous for the full extent of the deficiency in Def 1 (the smaller deficiency) through losses of the ring chromosome during somatic mitosis. The patterns of homozygous deficient tissues in plants with these two deficient rod chromosomes plus various combinations of ring chromosomes (R1, R2, two R2, R1 plus R2, two R1 plus one R2, two R2 plus one R1) have been compared and agree with expectancy on the basis of the cytological analysis of ring chromosome behavior in mitosis and the analysis of variegation (2 above) produced in plants with these same combinations of ring chromosomes.
8. The homozygous deficient tissues, lacking a locus for *Bm1*, have the phenotypic expression of *bm1* in their cell walls.
9. Plants homozygous for Def2 R2 have 22 chromosomes in their zygotes. Although the chromosome number has been increased, there has been no increase in the genome. Losses of the R2 chromosomes during development produce cells homozygous deficient for the extent of the deficiency in Def2. These cells are abnormal in appearance and are short lived, degenerating before maturity of the surrounding cells.
10. In plants with two deficient chromosomes and one or more compensating ring chromosomes, somatic alteration in constitution of a ring chromosome is reflected in modified tissues, having mutational characteristics. A number of repeatedly encountered, distinct types are briefly described. One type is indistinguishable from normal *bm1*.

## ACKNOWLEDGMENTS

Much of the investigation described in this paper was undertaken at CORNELL UNIVERSITY with the aid of a grant from the ROCKEFELLER FOUNDATION. The original material for this investigation was supplied by Dr. L. J. STADLER to whom the author is very grateful. To Dr. R. A. EMERSON of CORNELL UNIVERSITY the author is particularly indebted for his generosity in placing the facilities of the Department of Plant Breeding at her disposal and for his continued encouragement.

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# EFFECT OF THE *Dt* GENE ON THE MUTABILITY OF THE $a_1$ ALLELE IN MAIZE<sup>1</sup>

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Received April 8, 1938

## INTRODUCTION

THE mechanisms through which sudden changes in genes, that is, mutations, are produced have long been of interest to the geneticist. In attempting to obtain some insight into the nature of the mutation process, two methods of approach have been most commonly employed. One of these is the study and analysis of mutations induced by irradiation and the other the study of the so-called mutable genes or genes with high rates of spontaneous mutation. While there is no factual basis for believing that mutable genes differ from more stable ones save in their mutation rate, nevertheless there is the possibility that they constitute a distinct class in themselves and any conclusions reached about them might not be applicable to more stable genes.

In 1936, the writer described a dotted aleurone character in maize. Since that time the character has been studied in further detail and additional data obtained which have some bearing on the nature of gene mutation. These data show that the  $a_1$  allele, which has a low spontaneous mutation rate in the presence of recessive *dt*, becomes highly mutable with dominant *Dt*.

The character dotted aleurone appeared in a selfed ear of Black Mexican sweet corn obtained from Dr. L. F. RANDOLPH. The seeds on this ear occurred in the ratio of 12 self-colored:3 dotted:1 colorless seed. The dotted seeds had small dots or spots of aleurone color which were fairly uniform in size and distributed at random over the aleurone layer (fig. 1). Aleurone color is normally formed only when at least one dominant allele of each of the four primary factors,  $A_1$ ,  $A_2$ ,  $C$  and  $R$ , is present. Seeds homozygous for recessive  $a_1$  and possessing the dominant alleles of the other three primary genes have colorless aleurone. Strains of this constitution have been widely used in genetical experiments and are known as  $a_1$ -testers. As far as the writer is aware colored areas in the aleurone have never been found in these  $a_1$ -tester stocks. An examination of two thousand  $a_1$ -tester kernels with a low power binocular failed to disclose the presence of any colored cells. An analysis of the ear on which the dotted seeds

<sup>1</sup> Contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.



appeared showed that the 12:3:1 ratio came through the segregation of  $A_1 a_1$  and of a new dominant gene  $Dt$  which interacted with the recessive  $a_1$  to give the dotted character in three-fourths of the  $a_1$  class. The colorless seeds were homozygous for both  $a_1$  and  $dt$ . As the dotted class was homozygous for  $a_1$ , it would, therefore, normally have colorless aleurone.

The  $Dt$  gene was specific in its effect with  $a_1$  in that  $a_2$ ,  $c$  or  $r$  testers had colorless aleurone in the presence of  $Dt$ . The dominant  $Dt$  has been found only in the Black Mexican strain. All other strains of maize which have



FIGURE 1.—The dotted aleurone character is shown by all seeds on this ear which came from self pollinating a plant of  $a_1 a_1 Dt Dt$  constitution. Each colored area or dot represents a mutation of  $a_1$  to  $A_1$ . The small range in size indicates that mutations of  $a_1$  occur late in the development of the aleurone.

been tested have carried the recessive allele  $dt$ . Black Mexican sweet corn is normally homozygous for all the dominant alleles of the four aleurone factors and the detection of  $Dt$  in this line came through a mutation of an  $A_1$  allele to  $a_1$ . This  $a_1$  allele is apparently identical with the  $a_1$  allele found by EMERSON (1918) which is the source of all the  $a_1$  genes found in genetic stocks. It has not been possible to differentiate between these two  $a_1$  alleles on the basis of their interaction with  $Dt$ . Both of them produce colorless aleurone with  $dt$ .

EMERSON and ANDERSON (1932) found four alleles at the  $A_1$  locus. Two of them,  $A_1$  and  $A_1^b$ , give deep or strong colored aleurone,  $a_1^p$  produces a pale colored aleurone while  $a_1$  gives colorless aleurone. It was found that seeds homozygous for  $a_1^p$  occasionally, though rarely, had areas or dots of deep color in the pale colored aleurone. However, the frequency of the deep colored dots was no greater on seeds with  $Dt$  than on seeds with  $dt$ . It may be concluded, therefore, that the mutability of the  $a_1^p$  allele is not affected by  $Dt$ . The occurrence of the deep colored dots suggests that  $a_1^p$  may mutate to  $A_1$  but the frequency of such mutation is so low that it does not affect the data or the conclusions reached in this paper. However, seeds heterozygous for  $a_1^p$  and  $a_1$  show numerous deep colored dots on the pale background if  $Dt$  is present. The pale color is obviously produced by  $a_1^p$  and the dots of color by the interaction of  $a_1$  and  $Dt$ .

As the aleurone is triploid tissue, seeds possessing one, two, or three  $a_1$  alleles may be obtained by manipulating the dosage of the  $a_1$  gene. (See RHOADES 1936.) The mean number of dots per seed for each of the three classes was determined. As reported previously, the mean number of dots showed a linear relationship with the number of  $a_1$  alleles present. Additional data from several ears are given in table 1. As the pale-dotted and dotted seeds are borne at random on the same ear, they possess on the average identical genetic residua except for possible segregating genes linked with the  $a_1$  locus. The data in table 1 show that seeds of  $a_1^p a_1^p a_1 Dt Dt dt$  constitution which have one  $a_1$  gene have one-third as many dots as do sister seeds of  $a_1 a_1 a_1 Dt Dt dt$  heredity which have three  $a_1$  genes. Similarly  $a_1^p a_1 a_1 Dt dt dt$  seeds with two  $a_1$  genes have two-thirds as many dots as sister  $a_1 a_1 a_1 Dt dt dt$  seeds with three  $a_1$  genes. These data indicate that increasing the  $a_1$  dosage results in a proportional increase in the number of dots.

The results obtained when the  $a_1$  dosage is held constant while that of  $Dt$  is varied have previously been shown to be non-linear. This conclusion is supported by further data but this relationship will be discussed in a later section of the paper.

When the data showing the effect on the number of aleurone dots of varying the dosage of  $a_1$  and  $Dt$  were published (RHOADES 1936) nothing was known of the mechanism through which the colored cells were produced. One possible explanation was that the  $a_1$  allele, normally stable in the presence of  $dt$ , became unstable in the presence of  $Dt$  and mutated to dominant  $A_1$ . Each dot of color then would represent one mutation and the number of dots would represent the frequency of mutation while the size of the dot would indicate the relative time in ontogeny at which the mutation occurred. This hypothesis would account also for the dosage effect of  $a_1$  if it is assumed that the mutations to  $A_1$  of the several  $a_1$  genes occur independently of one another. As the  $A_1$  gene is known to affect anthocyanin formation in other tissues than the aleurone, experiments were planned which would determine the validity of this hypothesis and the present paper presents the results of these investigations.

#### EFFECT OF THE $Dt$ GENE ON PLANT AND PERICARP COLOR

In his classical study of plant colors in maize EMERSON (1921)<sup>2</sup> showed that purple plant color is conditioned basically by the interaction of three dominant factors,  $A_1$ ,  $B$ , and  $Pl$ . When recessive  $a_1$  is substituted for domi-

<sup>2</sup> EMERSON defines plant colors as "colors other than those related to chlorophyll, commonly in, but not limited to, such external plant parts of maize as the culm, the staminate inflorescence, the husks, the leaf sheaths, and to some extent the leaf blades. In contrast to this group are colors and color patterns related to chlorophyll or associated with the pericarp and the cob, the silks, the endosperm, the aleurone."

nant  $A_1$ , a brown color is produced. (SANDO and BARTLETT (1922) and SANDO, MILNER, and SHERMAN (1935) have shown that the purple pig-

TABLE I

*Mean number of aleurone dots on seeds with three  $a_1$  genes and seeds with two  $a_1$  genes from eight ears of crosses  $a_1 a_1 Dt Dt \times a_1 a_1^p dt dt$  or  $a_1 a_1 dt dt \times a_1 a_1^p Dt Dt$ .*

| PEDIGREE                  | MEAN NO.<br>DOTS ON<br>$a_1 a_1 a_1 Dt$<br>CLASS | NO. OF<br>SEEDS<br>IN<br>CLASS | MEAN NO.<br>DOTS ON<br>$a_1 a_1 a_1^p Dt$<br>CLASS | NO. OF<br>SEEDS<br>IN<br>CLASS |
|---------------------------|--|--------------------------------|--|--------------------------------|
| 2676(I) $\times$ 2511a    | 15.2   | 46                             | 10.0   | 48                             |
| 2500 $\times$ 2511b       | 9.0  | 32                             | 6.2  | 49                             |
| 4345(4) $\times$ 4344(I)  | 10.2   | 152                            | 6.1  | 172                            |
| 4345(6) $\times$ 4344(7)  | 7.4  | 61                             | 5.0  | 62                             |
| 4345(8) $\times$ 4344(I)  | 5.6  | 70                             | 4.2  | 76                             |
| 4345(9) $\times$ 4344(2)  | 11.5   | 16                             | 9.6  | 19                             |
| 4345(11) $\times$ 4344(2) | 3.5  | 39                             | 2.6  | 42                             |
| 4345(12) $\times$ 4344(8) | 2.9  | 20                             | 1.4  | 27                             |
|                           | 8 65.3   | 436                            | 8 45.1   | 495                            |
| Observed =                | 8.16   |                                | 5.64   |                                |
| Theoretical =             | 8.28   |                                | 5.52   |                                |
| on 3:2 ratio              |  |                                |  |                                |

*Mean number of aleurone dots on seeds with three  $a_1$  genes and seeds with one  $a_1$  gene from nine ears of crosses  $a_1 a_1^p Dt Dt \times a_1 a_1 dt dt$  or  $a_1 a_1^p dt dt \times a_1 a_1 Dt Dt$ .*

| PEDIGREE                  | MEAN NO<br>DOTS ON<br>$a_1 a_1 a_1 Dt$<br>CLASS | NO. OF<br>SEEDS<br>IN<br>CLASS | MEAN NO.<br>DOTS ON<br>$a_1 a^p a^p Dt$<br>CLASS | NO. OF<br>SEEDS<br>IN<br>CLASS |
|---------------------------|---|--------------------------------|--|--------------------------------|
| 2511 $\times$ 2500        | 11.6  | 50                             | 3.7  | 63                             |
| 2511a $\times$ 2500       | 6.6   | 43                             | 2.4  | 40                             |
| 4344(3) $\times$ 4345(5)  | 23.6  | 66                             | 7.0  | 62                             |
| 4344(4) $\times$ 4345(6)  | 13.3  | 59                             | 3.9  | 62                             |
| 4344(5) $\times$ 4345(6)  | 29.5  | 23                             | 12.0   | 26                             |
| 4344(6) $\times$ 4345(3)  | 20.8  | 38                             | 8.4  | 35                             |
| 4344(8) $\times$ 4345(12) | 27.5  | 26                             | 9.2  | 28                             |
| 4344(1) $\times$ 4345(4)  | 27.3  | 79                             | 8.3  | 83                             |
| 4346(7) $\times$ 4345(4)  | 20.3  | 55                             | 6.5  | 60                             |
|                           | 9 180.5   | 439                            | 9 61.4   | 459                            |
| Observed =                | 20.06   |                                | 6.82   |                                |
| Theoretical =             | 20.16   |                                | 6.72   |                                |
| on 3:1 ratio              |   |                                |  |                                |

ment is the anthocyanidin, chrysanthemin, while the brown pigment is the corresponding flavonol, isoquercitrin.) If the occurrence of colored areas in the aleurone of  $a_1 Dt$  seeds is caused by the mutation of  $a_1$  to  $A_1$ ,

we would expect to find on brown ( $a_1 B Pl Dt$ ) plants areas of purple representing tissue descended from cells in which a mutation from  $a_1$  to  $A_1$  had occurred. Therefore, a strain of maize of  $a_1 a_1 B B Pl Pl Dt dt$  constitution was synthesized. When plants of this strain were self-pollinated there resulted a ratio of 3  $Dt$ :1  $dt$  seed. Four hundred twenty-six plants were grown from seed with dotted aleurone and hence carrying  $Dt$ . They had the brown pigment characteristic of  $a_1 B Pl$  plants but in addition possessed from few to many narrow, longitudinal stripes of purple tissue on the husks and culms. The stripes were usually small, less than an inch in length, but occasionally one 6 to 8 inches long occurred. This small size indicated that the mutations to  $A_1$  occur relatively late in the development of the tissues. The 150 plants from  $dt$  seed had the same brown pigment as their  $a_1 B Pl Dt$  sibs but no stripes of purple tissue were found on these plants.

The study of the effect of  $Dt$  on the mutability of  $a_1$  in tissue of the culm and tassel was confined for the most part to brown plants of  $a_1 B Pl$  constitution since the brown pigment is distributed throughout the culm and sheaths and consequently offers a large area in which to detect mutations. However, the mutation hypothesis is subject to check in other plant color types. Plants of  $a_1 b Pl dt$  constitution are entirely green, whereas  $A_1 b Pl$  plants are also green except for purple colored anthers and some anthocyanin pigment at the base of the culm. A race of  $a_1 b Pl Dt$  individuals was synthesized. They had green culms and leaf sheaths but their green anthers had numerous small purple spots. Occasionally, though rarely, an entire anther was colored but the great majority of the colored areas were small, as was true in  $a_1 B Pl Dt$  plants. No attempt was made to find colored sectors at the base of the culm.

Plants with  $A_1 B pl$  constitute the sun red class. In this type anthocyanin is found in those portions of the culms, sheaths, and tassels which are exposed to light. Plants of  $a_1 B pl dt$  genotype possess no anthocyanin but  $a_1 B pl Dt$  individuals had the expected small colored regions in the culm, sheath, and anthers.

From the studies of the effect of  $Dt$  on aleurone and plant color it is seen that the production of the anthocyanin pigments can be interpreted on the assumption that  $a_1$  becomes unstable in a cell in which  $Dt$  is present. A further test of this mutable gene hypothesis is afforded by a study of pericarp color. EMERSON and ANDERSON (1932) have shown that red pericarp color is produced when the dominant  $A_1$  and the dominant  $P$  factors are present while a brown pericarp color is produced in  $a_1 P$  tissue. According to our hypothesis, ears on  $a_1 P dt$  plants should have brown pericarp with no red areas but those on  $a_1 P Dt$  plants should have red sectors on otherwise brown pericarp. This is precisely what was found. As in the

case of the experiments with aleurone and plant color, the size of the red sectors was small with the largest red area covering approximately one-third of a single kernel. Usually, the red sectors consisted of fine streaks running from the point of silk attachment towards the base of the kernel in a manner similar to the red stripes of variegated pericarp. In accordance with expectation no red sectors were found on  $a_1 P dt$  seeds.

The above data on the production of anthocyanin pigments in the three diverse tissues studied cannot be considered as proof of the mutation hypothesis even though the data are in agreement with such a hypothesis. A direct and conclusive test could be had only if a mutation occurred in sporogenous tissue which later gave rise to sex cells, enabling a genetic check to be made in subsequent experiments. Fortunately, such a test was possible.

It has been seen that  $a_1 B Pl Dt$  plants are brown with purple sectors. These purple areas are found in all parts of the plant which normally

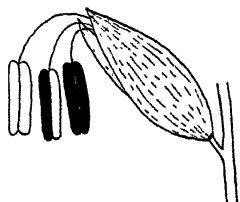


FIGURE 2.—A diagrammatic sketch of the three anthers of a single flower on a brown ( $a_1 B Pl Dt$ ) plant. One of the anthers is entirely purple, one is half purple and half green, and the third is wholly green. Tests of the constitution of the pollen in purple anthers show that in some cases some of the pollen grains have the  $A_1$  allele and some have the  $a_1$  allele. The  $A_1$  allele has been derived by a mutation of the  $a_1$  allele.

develop purple color when the plants are  $A_1 B Pl$ . EMERSON (1921) has shown that the purple pigment on  $A_1 B Pl$  extends into the anthers if the  $r^r$  or  $R^r$  alleles are present. The  $a_1 B Pl Dt$  stock used in these experiments had the  $R^r$  allele. Not infrequently  $a_1 B Pl Dt$  individuals had small purple sectors in the tassel. Sometimes (fig. 2) all three anthers of a flower were purple but often one anther was purple with the other two either green or sectorial. In some of the partly colored anthers one-half of the bipartite anther was purple and the other green; in others only a portion of one-half was colored with the remainder of the tissue green. Purple sectors of various sizes were found, however, and by far the most frequent class was that in which a small area on individual anthers was pigmented. It may be reasonably assumed that a mutation which occurred early enough to affect all of the cells composing the anther wall might also, in some cases at least, affect the sporogenous tissue. If, for example, a mutation from  $a_1$  to  $A_1$  occurred in a cell whose descendants comprised the tissue of the

anther wall and also the sporogenous tissue within the anther it would be expected that one-half of the pollen grains would carry  $A_1$  and the other half  $a_1$ . A ratio of 1 colored:1 colorless aleurone would be obtained when the pollen from such an anther was applied to  $a_1$ -tester silks. Varying ratios of colored:colorless seeds would be obtained if part of the sporogenous cells came from the cell in which the mutation occurred and part came from non-mutated cells. On the other hand, if there was no correlation between the genetic constitution of the cells of the anther wall and the sporogenous cells, or if the color of the anther resulted not from a mutation to  $A_1$  but through some effect which simulates such a change, no colored seeds would be found when the pollen from purple anthers was used in a cross with  $a_1 A_2 C R$  plants.

TABLE 2

*Summary of crosses with pollen from purple anthers occurring on  $a_1 B Fl Dt$  plants. Each purple anther listed came from a different plant. The  $Dt dt$  pair were segregating so that some of the ears had, in addition to colored seeds, only dotted or both dotted and colorless depending upon the  $Dt$  constitution.*

| COLOR OF ANTH                  | COLORED SEED | COLORLESS OR<br>DOTTED SEED | TOTAL |
|--------------------------------|--------------|-----------------------------|-------|
| Purple                         | 5            | 7*                          | 12    |
| Purple                         | 19           | 18*                         | 37    |
| Purple                         | 92           | 74*                         | 166   |
| Purple                         | 4            | 4                           | 8     |
| Purple                         | 2            | 49                          | 51    |
| Purple                         | 0            | 173                         | 173   |
| Purple                         | 0            | 110                         | 110   |
| Purple                         | 0            | 18                          | 18    |
| $\frac{3}{4}$ Purple           | 51           | 62                          | 113   |
| $\frac{1}{2}$ Purple           | 11           | 8*                          | 19    |
| $\frac{1}{2}$ Purple           | 13           | 34                          | 47    |
| $\frac{1}{2}$ Purple           | 8            | 114                         | 122   |
| $\frac{1}{2}$ Purple           | 1            | 67                          | 68    |
| $\frac{1}{2}$ Purple           | 0            | 142*                        | 142   |
| $\frac{1}{2}$ Purple           | 5            | 53*                         | 58    |
| Less than $\frac{1}{2}$ Purple | 2            | 36                          | 38    |

\* Only dotted seed.

In table 2 are listed the numbers of  $A_1$  and  $a_1$  seeds obtained when anthers either wholly or partly purple were tested in crosses with  $a_1$ -tester plants. Of the eight purple anthers used five produced seeds with colored aleurone. In four of the five cases the ratios suggest the presence of equal numbers of pollen grains carrying  $A_1$  and  $a$ , respectively. This 1:1 ratio is expected if the cell which gave rise to all of the sporogenous tissue became heterozygous for  $A_1$  following a mutation of one of the two  $a_1$  genes

to  $A_1$ . The fifth purple anther produced only 2  $A_1$  to 49  $a_1$  seeds. The deviation from a 1:1 ratio is too great to be accounted for by errors in sampling and indicates that the genetic constitution of the cells of the anther wall is not necessarily identical with that of all of the microspores. This reasoning is strengthened by the fact that three purple anthers produced no colored seeds when tested. It would appear that in ontogenetic development the separation of the sporogenous and anther wall tissue does not follow a precise pattern and that a mutation occurring in the cell giving rise to cells of the anther wall need not necessarily affect the sporogenous tissue. If the mutation occurs before the two cell lines have separated they will have the same constitution. The results from crosses using anthers sectorial for purple likewise show variation in  $A_1:a_1$  ratios and point to a similar conclusion.<sup>3</sup> The fact that colored seeds were produced when pollen from purple anthers was used is proof that  $a_1$  had mutated to  $A_1$  in the cells of the tested anther and it follows that the anthocyanin color produced in the aleurone, plant, and pericarp tissues resulted from similar mutations.

As individual anthers were used in making the crosses and the amount of pollen therefore was limited, the possibility of contamination was increased. An occasional colored seed might result from this contamination. However, the presence of the recessive mutant genes  $lg_1$ ,  $y$ , and  $su$  in the  $Dt$  stocks made it possible to check the origin of the colored seeds. In the tests made so far all colored seeds but one were the result of mutations to  $A_1$ .

Because the purple anthers were found on plants of  $a_1 B Pl Dt$  constitution the colored seeds produced by crossing with  $a_1$ -tester stocks gave rise to purple  $F_1$  plants as they were  $A_1 a_1 B b Pl pl$  while the colorless and dotted seeds produced brown ( $a_1 a_1 B b Pl pl dt dt$ ) and brown with purple stripes ( $a_1 a_1 B b Pl pl Dt dt$ ), respectively.

Three of the wholly purple anthers came from plants homozygous for  $Dt$ . In crosses with  $a_1$ -testers they produced only colored and dotted seeds (table 2). The dotted kernels came from  $a_1 Dt$  pollen and the colored seeds from pollen carrying  $A_1$  and, presumably,  $Dt$  unless the  $Dt$  gene is altered when it induces a mutation of  $a_1$  to  $A_1$ . If no change had occurred in the  $Dt$  gene the colored seeds were of  $A_1 a_1 Dt dt$  constitution. However, if  $Dt$  changes to  $dt$  coincidentally with a mutation of  $a_1$  the colored seeds will be  $A_1 a_1 dt dt$ . Some of these colored aleurone seeds were grown and self-pollinated. They yielded self-colored, dotted and colorless seeds in a 12:3:1

<sup>3</sup> It should be noted that rarely a self-colored seed is found on selfed ears of  $a_1 Dt$  constitution when bulked pollen is used. It seems probable that these self-colored seeds arise from the functioning of a gamete carrying an  $A_1$  allele derived by mutation from the  $a_1$  allele in either the sporogenous tissue or in the gametophyte.

ratio which is in accordance with the results expected from the selfing of  $A_1 a_1 Dt dt$  plants. If no dotted seeds had been produced the colored plants would have been  $A_1 a_1 dt dt$  and a ratio of 3 colored:1 colorless would have been obtained. These results argue that no change occurs at the  $Dt$  locus when a mutation of  $a_1$  to  $A_1$  occurs even though the  $Dt$  gene is directly responsible for the change in the  $a_1$  allele.

RELATIVE FREQUENCY OF MUTATION OF RECESSIVE  
 $a_1$  TO THE  $a_1^p$  AND  $A_1$  ALLELES

In the determination of the dosage effects of  $a_1$  the various levels of  $a_1$  were obtained by substituting the  $a_1^p$  allele for one or two  $a_1$  genes. It was possible to do this because the  $a_1^p$  allele is not affected by  $Dt$  and the mutations to  $A_1$  from  $a_1$  are clearly evident on the pale color produced by the  $a_1^p$  gene. The relative dosage effects of  $a_1$  would be subject to serious error if it were found that the  $a_1$  allele mutated to  $a_1^p$  with a considerable frequency because these changes would not be visible on the



FIGURE 3.—The kernel on the left has a large  $a_1^p$  area in which is found a smaller, deeper colored  $A_1$  dot. As  $a_1$  mutates both to  $a_1^p$  and  $A_1$  it is probable that one  $a_1$  gene mutated to  $a_1^p$  early in development and somewhat later a second  $a_1$  gene mutated to  $A_1$ . The rightmost kernel illustrates one of the mutations to  $A_1$  which occur infrequently at an early stage.

pale background produced by the  $a_1^p$  gene initially present since the intensity of color produced by one, two or three  $a_1^p$  genes is not noticeably different. It was therefore considered pertinent to determine the frequency with which  $a_1$  mutated to  $a_1^p$ . The dots on 2426 seeds of  $a_1 a_1 Dt$  constitution were examined with a low power binocular. There was a total of 80,955 dots of which 80 were  $a_1^p$  dots and the remainder  $A_1$  dots. The distinction between the two classes of dots was clear. The size of the  $a_1^p$  dots varied in the same manner as the  $A_1$  dots, that is, they were usually small but occasionally one was found covering a considerable portion of a seed. Such a mutation is shown in figure 3 where a pale colored sector is shown with an  $A_1$  dot clearly discernible in this area. It seems probable that in this seed one  $a_1$  allele mutated to  $a_1^p$  early in the development of



the aleurone and another  $a_1$  allele mutated several cell generations later to  $A_1$  to give the deeper colored spot.

It is apparent that the conclusions reached on the effects of different dosages of the  $a_1$  allele are not seriously affected by the occurrence of  $a_1^p$  mutations.

Interestingly enough the  $a_1^p$  allele has been found only once in the great numbers of strains of maize collected in different parts of the world. Apparently mutations to this allele have occurred infrequently in the past. In these investigations the mutations from  $a_1$  to  $A_1$  occurred about 1000 times as frequently as the mutations to  $a_1^p$ .

There exists a fourth allele,  $A_1^b$  at the  $a_1$  locus. The  $A_1^b$  allele is indistinguishable from  $A_1$  in its effect on plant and aleurone color but produces a dominant brown pericarp with the  $P$  gene. No tests have been completed as yet to determine the relative frequencies of  $A_1$  and  $A_1^b$  mutations. There is also a possibility that some of the mutations may be to new, previously unknown, alleles.

As reported in table 1 of the writer's 1936 paper  $A_1 a_1 C R A_2 Dt$  seeds have deep self color with no evidence of dots. If  $A_1 a_1 a_1$  aleurone was lighter than  $A_1 A_1 a_1$  we would expect in  $A_1 a_1 a_1 Dt$  aleurone to find areas or dots of a deeper color which would represent cells in which one of the  $a_1$  alleles had mutated to  $A_1$ . However, it has not been possible, at least in the writer's experience, to detect a difference in intensity of aleurone color in seeds possessing one, two or three  $A_1$  alleles.

While the  $a_1$  allele is highly unstable with  $Dt$  there is no indication that the  $A_1$  allele derived by mutation from  $a_1$  is unstable either with  $Dt$  or  $dt$ . Purple plants of  $A_1 a_1 B Pl Dt$  constitution, the  $A_1$  allele being one derived by mutation, do not show the brown colored sectors expected if the  $A_1$  gene mutated back to  $a_1$ . These observations are not extensive but there are, at present, no indications that the  $A_1$  mutations from  $a_1$  are unstable. While more data should be had on this point, it can be unequivocally stated that if  $Dt$  does affect the stability of reverted  $A_1$  it does so with a low frequency.

#### GENES MODIFYING THE MUTATION FREQUENCY

The data in table 1 show considerable variation in the mean number of dots on the kernels from different ears possessing the same genetic constitution for  $a_1$  and  $Dt$ . It is possible to ascribe these differences to certain genetic factors which modify the frequency with which the mutations to  $A_1$  occur. While it appears likely that various stocks have different sets of lesser modifiers certain data were obtained which indicate that a single locus exerted a major effect on the frequency of mutation. Selfed ear 4347-7 was homozygous for  $a_1$  and  $Dt$ . The  $a_1$  alleles in this ear are de-

scendants of a common  $a_1$  allele. The other aleurone genes were homozygous dominant so every seed on the ear was dotted. The number of dots on each of the 154 seeds was determined and the data represented graphically as a rectangular histogram. The mean number of dots per kernel was 31.1. The range was 93. As figure 4a shows, there was an indication of bimodality suggestive of a 3:1 ratio for a dominant modifier  $M$  which decreases the number of dots. To test this hypothesis, kernels were selected which had a high number of dots. Theoretically they should be  $a_1 a_1 Dt Dt m m$ . Seeds with an intermediate number close to the mean of 31.1 dots were also selected and planted. They should be  $a_1 a_1 Dt Dt M m$ . Finally, seeds possessing a low number of dots were picked. On the further assumption that the  $M$  factor only partially inhibits the mutation frequency and that there is a cumulative effect by increasing the dosage of  $M$  these seeds should be  $a_1 a_1 Dt Dt M M$ . Plants from these three classes of seed were grown and self-pollinated and the number of dots on each individual seed from ears in each of the three classes was determined. The seeds in the high class should, on the theory, give rise to offspring with a high mean number of dots and should give a unimodal frequency distribution since they would be homozygous for  $m$ . Figure 4b is representative of the results obtained with three selfed ears of this class. The mean number of dots is somewhat higher than on the parent seed but there is no tendency toward a bimodal distribution. Although it was desirable to have frequency distributions for additional ears of this class, the amount of labor involved in counting the dots on seeds with high numbers discouraged the accumulation of more data. However, an inspection of the other ears of this class indicated that they were of the same type as the three which were selected at random for classifying. Likewise, the ears from seeds with a low number of dots had uniformly a low average number of dots in the offspring and the data when plotted as a histogram showed no indication of a bimodal distribution. Figure 4c is representative of one ear of this class. They all had a unimodal distribution. Obviously the critical class for the hypothesis is the group with the intermediate number of dots. Individuals from this group should yield progeny with a frequency distribution similar to that obtained for the kernels of the parent ear 4347-7. Seeds from five ears of this class were classified for dot number and the data plotted. Figure 4d is typical of the results. They had frequency distributions closely resembling that of the parent ear. Furthermore, approximately one-fourth of the kernels fall into that portion of the histogram which presumably includes the  $m m$  class.

The difference in the mean number of dots between members of the  $M M$  class, for example, may be attributed to the action of numerous modifying genes whose effect, however, is relatively slight in comparison

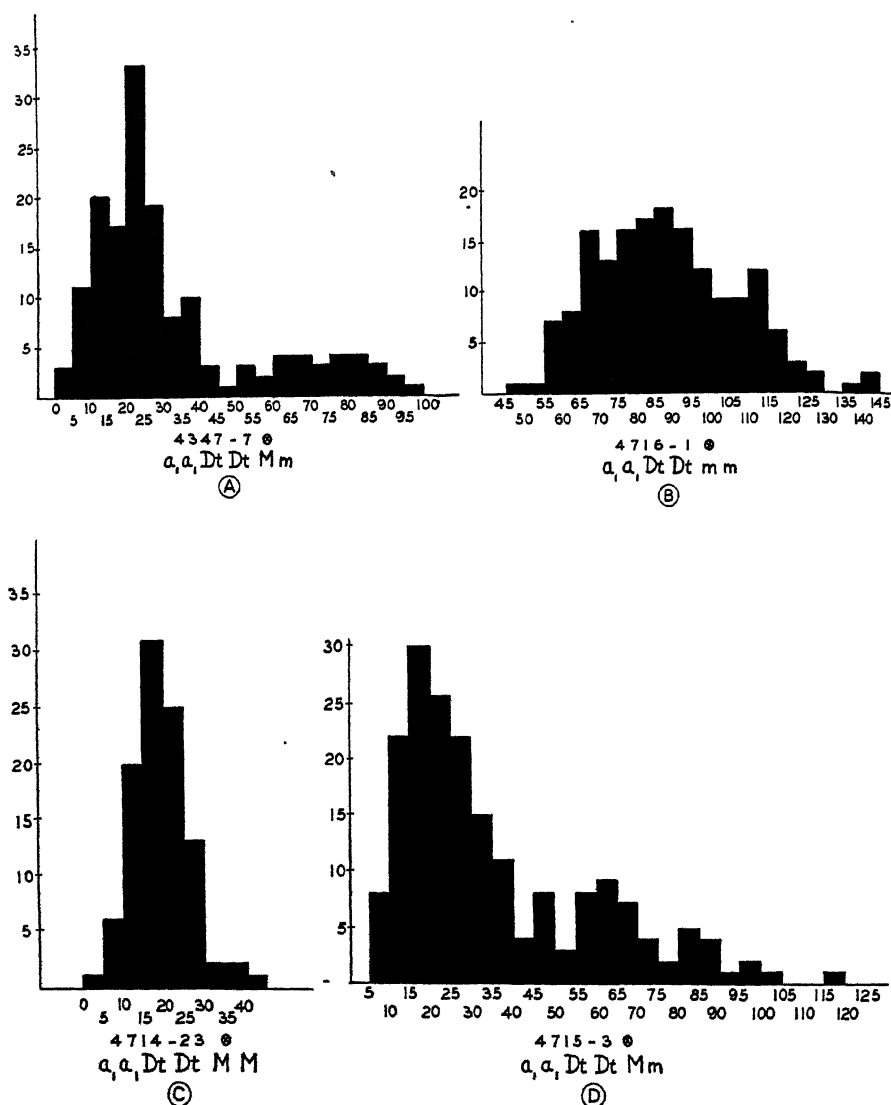


FIGURE 4.—Frequency histograms showing effect of  $M$  and  $m$  genes on the mutation rate of  $a_1$ . Number of dots per seed is plotted on the abscissa and the number of seeds on the ordinate. Figure 4a represents the data from selfed ear 4347-7 in which the presence of the  $M$  and  $m$  genes was suspected from the bimodality of the distribution. The data from selfed ear 4716-1 are shown in Figure 4b. The unimodality of the histogram and the high average number of dots suggest that the  $m$  gene is homozygous. The data from selfed ear 4714-23 is illustrated in Figure 4c. The homozygosity of the  $M$  factor is indicated by the unimodality of the histogram and the low average number of dots. Figure 4d from the data of 4715-3 has the bimodality expected upon selfing  $M m$  plants.

to the  $M m$  pair. Further work is necessary to prove the existence of these lesser modifiers but the material seems admirably suited for such a demonstration. In figure 5 the pedigree of the  $Mm$  line is shown. It is not certain which parent in the original cross brought in the  $M$  or  $m$  alleles.

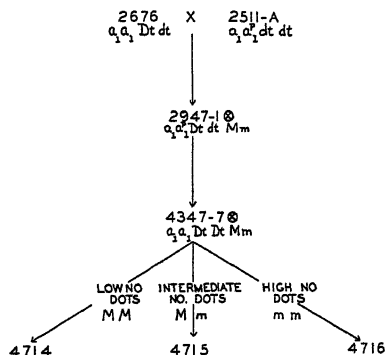


FIGURE 5.—Pedigree of ear 4347-7 in which the presence of the  $M$  and  $m$  alleles, which modify the effect of  $Dt$  on  $a_1$ , was first detected.

In table 3 the number of dots on each seed planted in families 4714-4716 is shown in column 2. There was a tendency for the mean number of dots on the seed of the progeny to exceed the number on the parental seed. Plant 4347-7 was grown under adverse conditions in the summer of 1936 at Ames, Iowa, while families 4714-4716 were grown under favorable conditions in 1937 at Arlington Experiment Farm, Arlington, Virginia. Whether or not the environment plays a role in the mutation frequency is unknown but these data suggest such a possibility.

#### LINKAGE RELATIONS OF THE $Dt$ GENE

The four primary genes for aleurone color development,  $A_1$ ,  $A_2$ ,  $C$  and  $R$ , are located in different chromosomes. No indication has been found that  $Dt$  is linked with  $A_1$ ,  $A_2$ , or  $R$ . There are some data which show linkage of  $Dt$  with  $C$  and suggest that  $Dt$  belongs in chromosome IX. When  $A_1 a_1 C c Dt dt$  plants are selfed a 36:9:19 ratio is expected with independence of the three segregating factors. Previous data of this nature closely approached this ratio but the average number of dots per kernel in these ears was low so that seeds genotypically  $Dt$  might be phenotypically colorless or  $dt$ . Therefore the test of linkage between  $Dt$  and  $C$  was repeated using a strain in which there was a much higher number of dots. With  $Dt$  and  $C$  in coupling phase a 9:3:4 ratio is expected with complete linkage of  $Dt$  and  $C$  as contrasted with the 36:9:19 ratio expected with independence. There resulted in  $F_2$  from the selfing of  $A_1 a_1 C-Dt c-dt$  individuals

1761 colored:501 dotted:792 colorless seeds. The ratio of dotted and colorless seeds is far from a 9:19 ratio and approaches a 3:4 ratio. Any errors in classification should favor the colorless class at the expense of the dotted class. The *wx* gene was segregating in the above ears and gave a close fit to a 3:1 ratio. Since *wx* was brought in with *c*, with which it is linked, the excess of *Dt* seeds is probably not due to a failure of a portion of the *c-dt* gametes to function. However, if *Dt* is independent of *C* the

TABLE 3

*Data on average number of aleurone dots per seed from selfed ears of M M, M m and m m constitution. The three families are descendants of selfed ear 4347-7.*

| PLANT    | NO. DOTS ON<br>PARENTAL<br>SEED | INFERRED<br>CONSTITUTION | NO. SEEDS<br>COUNTED | MEAN NO.<br>DOTS | TYPE OF<br>FREQUENCY<br>DISTRIBUTION |
|----------|---------------------------------|--------------------------|----------------------|------------------|--------------------------------------|
| 4714(2)  | 12                              | <i>M M</i>               | 75                   | 16.4             | unimodal                             |
| 4714(9)  | 9                               | <i>M M</i>               | 100                  | 8.8              | unimodal                             |
| 4714(12) | 9                               | <i>M M</i>               | 200                  | 16.8             | unimodal                             |
| 4714(13) | 11                              | <i>M M</i>               | 75                   | 11.4             | unimodal                             |
| 4714(14) | 15                              | <i>M M</i>               | 75                   | 19.6             | unimodal                             |
| 4714(23) | 15                              | <i>M M</i>               | 101                  | 18.9             | unimodal                             |
| 4714(24) | 19                              | <i>M M</i>               | 100                  | 16.8             | unimodal                             |
| 4715(7)  | 31                              | <i>M m</i>               | 158                  | 31.7             | bimodal                              |
| 4715(3)  | 28                              | <i>M m</i>               | 193                  | 34.4             | bimodal                              |
| 4715(22) | 30                              | <i>M m</i>               | 168                  | 39.9             | bimodal                              |
| 4715(10) | 36                              | <i>M m</i>               | 124                  | 38.2             | bimodal                              |
| 4715(5)  | 31                              | <i>M m</i>               | 100                  | 33.8             | bimodal                              |
| 4716(1)  | 72                              | <i>m m</i>               | 169                  | 87.6             | unimodal                             |
| 4716(24) | 68                              | <i>m m</i>               | 106                  | 83.9             | unimodal                             |
| 4716(3)  | 66                              | <i>m m</i>               | 125                  | 76.9             | unimodal                             |

excess of dotted kernels could be explained by a gametophyte factor linked with *dt*. Linkage tests with other genes in chromosome IX, with *C* homozygous, will tell if the *Dt* factor belongs in this group. The *Dt* gene has failed to show linkage with any other tested genes which include from one to four members in each of the other nine groups.

Earlier data which gave a faint suggestion that the number of dots, that is, the mutation rate, was influenced by the dosage of *C* were not substantiated by more extensive tests. A similar conclusion is true for the *R* gene. These results are in accord with the mutation hypothesis. The dosage effect of *A*<sub>2</sub> was not determined but it should prove to be in the same category as *C* and *R*.

Self-colored aleurone depends upon the interaction of a set of complementary factors. This is also true for the dotted character since the colored dots are produced only when *a*<sub>1</sub> mutates to *A*<sub>1</sub> and the other mem-

bers of the complementary set are represented by a dominant allele. A strain was obtained which was  $a_1 a_1 C C R R Dt Dt$  and  $A_2 Bt/a_2 bt$ . Selfed plants of this genotype gave a ratio of 3 dotted to 1 colorless seed since the  $A_1 a_2$  pair was segregating. The brittle endosperm ( $bt$ ) gene showed 8 percent recombination with the dotted character. This would seem to indicate strong linkage between  $Dt$  and  $bt$ . Actually the linkage is between  $A_2$  and  $bt$ . As the  $A_2$  allele must be present before mutations of  $a_1$  to  $A_1$  can produce the dotted character most of the  $Bt$  seeds were dotted and most of the  $bt$  seeds were colorless. The 8 percent recombination value found for  $Dt$  and  $bt$  is the map distance between  $A_2$  and  $bt$ . This example of pseudo-linkage suggests that certain linkages found by one investigator and not confirmed by another may be due to a similar genetic set-up in which one of the characters is determined by complementary genes and different members of the set are segregating in the two tests.

#### DISCUSSION

A consideration of the data presented in this paper as they relate to the behavior of unstable genes reported by other investigators shows several points of similarity as well as difference. Furthermore, these data have some bearing on the nature of gene mutation, especially since GOLDSCHMIDT (1938) has recently promulgated the revolutionary view that mutations are a consequence of position effects.

It has been held, most recently by GOLDSCHMIDT, that mutable or unstable genes constitute a class distinct from stable genes; that their relatively high mutation rate springs from some peculiarity in their structure and, therefore, that any conclusions reached with mutable genes concerning the nature of gene mutation might not be applicable to the mutation phenomenon in general. Inasmuch as mutable genes offer many advantages for the study of the mutation process it is desirable to ascertain if there is a fundamental difference between stable and unstable genes.

Let us see how the data in this paper bear on this question. The  $a_1$  allele has been shown to be an extremely stable gene when  $dt$  is present but is highly mutable when the  $Dt$  allele replaces  $dt$ . The classification of the  $a_1$  gene as mutable or stable depends, therefore, upon the presence or absence of the  $Dt$  gene. Whether or not the behavior of all so-called mutable genes is caused by a similar genetic situation cannot be stated at this time but the definite effect of  $Dt$  on the mutation frequency of  $a_1$  certainly suggests that in some cases at least the difference between stable and unstable loci may be more apparent than real and results from the genetic environment rather than from some intrinsic dissimilarity.

Data have been presented in a previous section which indicate that the effect of  $Dt$  on the mutation rate of  $a_1$  is influenced by the dominant gene

$M$  and that there are also a number of lesser modifying genes. Somewhat comparable results have been reported by various workers with unstable genes. DEMEREC (1929) found three genes which affected the mutation frequency of unstable miniature-3 alpha and gamma of *Drosophila virilis*. DEMEREC also found one factor which increased the mutability of miniature-3 alpha in the germ cells. EMERSON (1929), HERTWIG (1926), and KIHARA (1932) have found the mutation rate of unstable loci to be markedly influenced by the presence of certain genes. These cases may not be strictly comparable with the effect of the  $M$  gene. Here we have a situation where the mutability of the  $a_1$  allele is primarily controlled by  $Dt$  but the influence of  $Dt$  on the  $a_1$  allele is to some extent modified by other genes. The  $a_1$  allele is stable with  $dt$  irrespective of the presence or absence of these modifying genes. In the cases cited above the modifiers presumably affect the unstable loci directly.

The evidence that the mutability of certain loci is controlled, at least to some extent, by other genes, suggests that the relative mutation rate of different loci may differ according to the presence or absence of specific modifiers.

The size of a colored sector carrying  $A_1$  is a fair indication of the relative time during ontogeny at which the mutation occurred, that is, larger colored areas descend from earlier occurring mutations than do smaller ones. The mutation rate at all stages of development of any tissue can be determined by a statistical study of the frequency with which areas of different size are found. Such a statistical study has not yet been made for the  $a_1$  gene, but the rare occurrence of large colored regions on dotted kernels and the fact that the size range among the aleurone dots is relatively small (fig. 1) indicates that in the aleurone the mutations of  $a_1$  occur predominantly in the later stages of development. The purple stripes on the culms and sheaths of brown ( $a_1 B Pl Dt$ ) plants and the red sectors in the pericarp of  $a_1 P Dt$  plants had a size range which indicated that in these tissues also the great majority of the mutations occurred late in development. If mutations to  $A_1$  took place consonantly throughout all tissues of the plant irrespective of the ontogenetic age of the different tissues we would expect to find many seeds with self-colored aleurone, since the culm has completely matured before fertilization, and mutations in the sporogenous tissue concomitant with mutations in the culm would give rise to groups of colored seeds on the ear. Inasmuch as the three tissues mature at different times and mutations occur late in the development of each tissue the possibility is indicated that the physiological age of the tissue is in some way concerned with the mutability of the  $a_1$  gene.

Mutation rates for various unstable loci have been determined by several investigators. ANDERSON and EYSTER (1928) found that the rate of

change of the unstable variegated gene in maize pericarp increases toward the end of development of the pericarp. DEMEREC (1931) found that the unstable lavender gene of Delphinium had a high mutation rate in early and late stages but had a low rate in intermediate stages. IMAI (1934) reported a similar situation with yellow-inconstant-1 and flecked in Pharbitis. DEMEREC, however, states that the unstable rose gene of Delphinium mutates with constant frequency throughout all stages of development while, in direct contrast, the reddish-alpha gene of *Drosophila virilis* (DEMEREC 1928) mutates only at the maturation division in heterozygous females. If the mutability of unstable genes is conditioned by a peculiar physiological state, it seems that for the several mutable loci the threshold value of this state is reached at different stages in development.

The characteristic direction of mutation of unstable genes is from the recessive to the dominant, wild type allele. One exception is found in Pharbitis (IMAI 1934) where the unstable willow-leaf gene mutates to the maple-leaf allele which is dominant to willow-leaf but recessive to wild type. Recently DEMEREC and SLIZYNSKA (1937) reported an unstable cream allele, at the white locus of *Drosophila melanogaster*, which mutated both to the cherry and wild type alleles. The  $a_1$  allele changes to  $a_1^p$  and  $A_1$  but the frequency of mutation to the  $A_1$  allele is a thousand times greater than that to  $a_1^p$ . In addition to mutating to the wild type allele, DEMEREC found that his unstable miniature genes in *Drosophila virilis* changed occasionally from one unstable allele to another. DEMEREC's (1935) citation of the change of the near-self type to dark-crown variegation in maize pericarp as a case of a mutation of one unstable allele to another is erroneous. According to EMERSON, the difference between the heritable near-self and non-heritable dark-crown types is that in the near-self the mutation to red occurs in a sub-epidermal cell which develops into germinal as well as pericarp tissue, while in the dark-crown type the mutation occurs in epidermal tissue and the germ line is unaffected. We have obtained no evidence that the  $a_1$  gene mutates to alleles varying in their response to *Dt* but they would be difficult to detect unless they occurred in a strain homozygous for all modifying genes.

As the aleurone is triploid tissue it was possible to have one, two or three  $a_1$  genes present and the mutation rate was found to be proportional to the dosage of  $a_1$ . This increase in mutation rate with increase in number of genes able to mutate is in contrast with EMERSON's results with variegated pericarp of maize in which he found the mutation rate of the variegated gene was greater in the heterozygous than in the homozygous condition. EMERSON considered the increased mutability in the heterozygous condition to be caused by modifying genes carried in the homologous chromosome. A similar result would be expected in the present study if



dominant modifiers for increased mutability of  $a_1$  were located in the  $a_1^p$  chromosome.

Cytological observations of microsporocytes of plants carrying *Dt* in the heterozygous condition have shown no chromosomal abnormalities. An examination of the pachytene stage of plants heterozygous for a dominant  $A_1$  allele derived by mutation shows the chromosomes III to be normal in appearance. Likewise, chromosome IX of a *Dt* stock has no detectable structural change. The writer is not willing to state that a more intensive study might not reveal some minute difference between *Dt* and *dt* lines in the structure of the chromosomes, but there is no evidence of such at present and it can be stated that there are no gross structural differences in the *Dt* and *dt* lines. Further, there is no difference in the "stainability" of the chromosomes in *Dt* and *dt* stocks (SCHULTZ 1936).

As a result of his extensive studies with mutable genes, DEMEREC has advanced the view that changes or mutations in unstable genes are produced by a chemical process rather than through some mechanical shifting or rearranging of chromatin. He visualizes an unstable gene as one with a molecular group in a chemically labile state. DEMEREC also believes that there is no clear cut difference between stable and unstable genes. The data on  $a_1$  and *Dt* interrelationships show clearly that this latter statement is true at least for this one example. Whether or not the  $a_1$ -*Dt* data can be held to support the theory that mutations of unstable genes are caused by chemical processes (for example, changes in the side chains of gene molecules) it is reasonably certain that they cannot be interpreted by the theories of the nature of variegation advanced by PATTERSON (1932), STERN (1935), and SCHULTZ (1936). The writer does not contend that these theories are inadequate to account for specific cases of variegation, but it is becoming evident that no one theory can satisfactorily explain the diverse types of variegation any more than one theory of gene mutation can account for all the different phenomena which are categorically called mutations. It is true that somatic crossing over (STERN), loss of a part of a chromosome carrying the dominant allele (CLAUSEN 1930, McCLINTOCK 1932, PATTERSON), are responsible for certain cases of variegation but account only for the appearance of a recessive character that was present in a heterozygous condition. In the great majority of unstable genes the direction of mutation is from the recessive to the dominant. It is difficult, at least for the writer, to see how any mechanical theory as loss or rearrangement of chromosome pieces can account for the high mutability of  $a_1$  in the presence of *Dt*. The following facts seem to negate such an explanation: (1) There is no visible chromosomal aberration as a translocation or ring chromosome in the *Dt* line. (2) The *Dt* gene remains unaltered when a mutation of  $a_1$  takes place. On the theory of a rearrange-

ment of chromatin involving the  $a_1$  and  $Dt$  loci we would expect a 1:1 correspondence between mutations of the  $a_1$  allele and mutations of the  $Dt$  allele. (3) The dosage effect of  $Dt$  is not arithmetic as was true for the  $a_1$  gene but is exponential. Numerous biochemical reactions such as the effect of a change of pH on enzyme activity offer an interesting parallel to the effect of changing the dosage of  $Dt$  if the possibility is considered that the  $Dt$  gene produces some chemical substance that accelerates the mutation rate of  $a_1$  and that the amount of this substance produced by two  $Dt$  genes as compared with one causes the mutation rate to be more than twice as rapid. (4) Mutations of  $a_1$  occur to at least two different alleles. (5) The loci of  $Dt$  and  $a_1$  show independence in inheritance.

It also is difficult to reconcile the above statements with GOLDSCHMIDT's view that mutations are position effects. Some dotted strains average over 200 dots or mutations per seed. On the position effect hypothesis every mutation of  $a_1$  represents some kind of rearrangement at or near the  $a_1$  locus. In the high dotted strains this would mean that breakages at this locus would be extremely frequent. It is difficult to visualize a mechanism by which a gene in one chromosome could produce so many breaks at or near a specific locus of another chromosome. Furthermore, unless all rearrangements are of a small intra-chromosomal type, evidence of gross structural changes should be found. The data offer no support for the position effect hypothesis as the cause of the mutability of  $a_1$  in the presence of  $Dt$ .

The  $Dt$  gene increases, apparently, only the mutability of the  $a_1$  allele. The mutation rates of other loci may be increased but studies made with the recessive aleurone genes,  $a_2$ ,  $c$ , and  $r$ , and the recessive endosperm genes,  $wx$  and  $su$ , have shown no effect of  $Dt$  on the mutability of these loci. The specificity of  $Dt$  in affecting only the  $a_1$  allele is in contrast to the results of DEMEREC (1937) and PLOUGH and HOLTHAUSEN (1937), who reported a general increase in mutation frequency in certain stocks of *Drosophila* where a mutability factor was present.

#### SUMMARY

1. The  $a_1$  allele is a stable gene in the presence of  $dt$  but becomes highly mutable with dominant  $Dt$ . This pronounced influence of one gene upon the mutability of another suggests there may be no fundamental difference between stable and unstable genes.

2. The mutation frequency is proportional to the dosage of the  $a_1$  gene.

3. Mutations of  $a_1$  to the  $A_1$  allele occur about a thousand times as frequently as to the  $a_1^p$  allele.

4. Mutations of  $a_1$  occurred in the aleurone, culm and sheaths, and pericarp. The size of the mutated areas indicates that mutations of  $a_1$

occur late in the development of each tissue. The evidence indicates that mutations do not occur consonantly in all parts of the plant.

5. Certain data indicate the existence of a dominant modifying gene which decreases the mutation frequency.

6. The  $a_1$  and  $Dt$  genes are not linked. The  $a_1$  gene is in chromosome III and  $Dt$  may be in chromosome IX.

7. Cytological observations of the  $Dt$  and  $dt$  stocks show no evidence of gross chromosomal aberrations.

8. The data do not support the view that mutations of  $a_1$  induced by  $Dt$  result from a position effect. The  $Dt$  gene remains unaltered when a mutation of  $a_1$  takes place.

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# STUDIES ON SIZE INHERITANCE IN MICE<sup>1</sup>

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Received February 5, 1938

IN RECENT years specific evidence has accumulated for the existence of genes influencing body size in mice. GREEN (1931 et seq.) crossed two species of mice differing greatly in body measurements in order to test for possible linkage between color genes and size genes. He reported association in  $F_2$  and backcross populations of larger size and the recessive gene brown, introduced by the larger parent. Two other chromosomes marked with the dilution and non-agouti genes at first showed association with larger body size, but subsequent data indicated that the differences were statistically significant only as regards the chromosome carrying brown. GREEN concluded that the chromosome bearing this gene also contained genes influencing body size. Later (GREEN 1935b) data were reported indicating crossing over between brown and size genes located on the same chromosome.

CASTLE, GATES and REED (1936) repeated the experiments of GREEN using different inbred strains of mice. They reported significant differences in body length and body weight for brown mice over black mice and dilute mice over intense mice. Further studies by CASTLE, GATES, REED and LAW (1936) gave the following interesting results. Brown mice were significantly larger than their black sibs by all size criteria used. Dilute mice were regularly smaller than their intense sibs. However, unlike the previous backcrosses studied, the dilution gene was closely associated with the gene for short-ear. Pink-eyed segregates were slightly smaller than their normal sibs, although the pink-eye gene was originally introduced into the cross by the larger parent. Linkage with size genes seemed to be an inadequate explanation for the size differences found in these backcross populations. A direct, physiological action on growth by the mutant genes studied was the interpretation given. As regards their influence on size, brown and dilution were found to have accelerating effects, pink-eye and short-ear retarding effects. Only in the case of the dilution and short-ear genes, however, was there direct evidence for assuming that these genes were themselves influencing body growth.

The crosses to be reported here were a continuation of the program begun at the BUSSEY INSTITUTION. Several objectives were in mind in car-

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rying out this work. First, an attempt was made to test for genes influencing size in the chromosome marked by recessive (piebald) spotting (*s*) and by waltzing (*v*). Second, further data relating to the association of larger body size and brown were desired since indirect evidence suggested that this was an effect of the brown gene itself on growth. In one cross made in this series, involving the Pincus inbred strain, the chromosome bearing brown is introduced from a source different from those already tested. In a second cross, using the wild *musculus* strain, a different genetic set-up is used in an attempt to determine the characteristics of this association. Third, the effects of dilution and short-ear were obtained from crosses in which either the chromosome bearing only dilution or the chromosome bearing both dilution and short-ear closely linked were under consideration. Backcross offspring segregating for short-ear (free of the dilution gene) are considered in this study in the hope of arriving at some estimation of the direct influence of this gene. The fourth objective relates to a study of the apparent localized effect of the dilution gene in the tail region which was reported in a previous study (CASTLE et al 1936 II).

The inbred strains of mice used were the Little (*dba*), *bactrianus*, Gates, and Japanese waltzing strains described in the previous reports. In addition a brown, piebald inbred strain designated the Pincus strain, a wild *musculus* (*DBA*) strain and an inbred strain of mice homozygous for short-ear (*se*), and extreme dilution (*c<sup>h</sup>*), an albino allele, were used. This is designated the Snell strain. A close approach to homozygosity is expected in all races employed except the last two which unfortunately are not as highly inbred as desired.

Size characters studied were adult body weight, body length and tail length measured in the manner described by SUMNER (1927). In addition tail ring number, number of caudal vertebrae, and length of 15th caudal vertebra were used as size criteria in some crosses.

In backcrosses designed to test for association between size genes and specific qualitative genes the segregates are treated separately as to type, considering one pair of alleles at a time. For example, all mice of backcross matings carrying the dominant gene black (*B*) have been compared with all recessive brown (*b*) mice, weighted equally. In like manner, the agouti (*A*) offspring have been compared with all non-agoutis (*a*), intense (*D*) mice with dilutes (*d*), etc. Sexes have been treated separately. Differences between the means with their corresponding standard errors, along with the general trend of the mean differences, have been used as criteria for the detection of association. In crosses where the parental strains used are nearly the same size, and where the number of backcross offspring obtained was comparatively small, the data have been subjected to

analysis of variance (after SNEDECOR 1934). This entails the use of the sum of squares of combined data. In this manner the variation traceable to specified sources may be segregated. For testing significance between classes the mean squares and a standard error derived from the residual or experimental error is used.

#### CROSS I

Backcross of Gates females to males of the  $F_1$  (Gates/Japanese) race

There are five markers in the chromosomes of the Gates strain of mice used in this cross, pink-eye ( $p$ ), short-ear ( $se$ ), dilution ( $d$ ), brown ( $b$ ), and non-agouti ( $a$ ). The genes  $d$  and  $se$  are in the same chromosome and are closely linked. This race was obtained originally by Dr. W. H. GATES by crossing a dilute, pink-eye mouse of Strong's inbred strain to Little's (dba) strain. In body weight 17 adult males range from 22 to 33 grams, an average weight of 26.2 grams.

The Japanese waltzer, which probably originated from the common mouse of Central Asia, bears the varietal name of *Mus wagneri rotans* Fortuyn. These mice were homozygous for piebald ( $s$ ), waltzing ( $v$ ), black ( $B$ ), intensity ( $D$ ), and non-agouti ( $a$ ). The average weight of males was found to be 17.6 grams and of females 16.8 grams. Other measurements obtained from a small number of mice were body length 8.0 cms and tail length 8.2 cms.

The  $F_1$  animals were secured from matings of ♀ Gates by ♂ Japanese. Heterosis was quite apparent as regards body weight, which for the combined sexes was 24.8 grams. Other measurements which closely approach those of the larger Gates strain are body length 9.4 cms and tail length 8.2 cms.

Since  $d$  and  $se$  are closely linked there are eight backcross classes, brown, black, pink-eye brown, pink-eye black, dilute brown short-ear, blue short-ear, pink-eye dilute brown short-ear, and pink-eye blue short-ear.

Comparative size measurements for each pair of alleles under consideration are given in table 1. The results are similar to those obtained by us in the reciprocal cross using  $F_1$  animals as mothers. Brown is unmistakably associated with larger body size as determined by a series of three size measurements. Dilute short-ear mice are smaller than their intense, normal ear sibs in body weight and tail length measurements. In other backcrosses studied dilution was found to be regularly associated with larger body size. The pink-eye gene although derived from the larger parent had a tendency to be associated with smaller body size as reported in the reciprocal backcross (Castle et al 1936 II). This tendency was manifested in all characters studied except body weight in the case of females.



In this backcross, using Gates mothers, the association is evident only in body length and tail length measurements, whereas greater body weight for both sexes is associated with the pink-eye gene. Although the sample obtained here is not as large as that reported for the reciprocal cross, the data do not clearly indicate a definite association between the pink-eye gene and smaller body size.

## CROSS 2

The ♀  $F_1$  (Gates/Japanese) by ♂ Japanese backcross

This cross was made in order to determine whether a correlation exists between piebald (*s*) and waltzing (*v*), located on separate chromosomes,

TABLE 1

*Comparative size measurements of different phenotypic classes from matings of ♀ Gates by ♂  $F_1$ .*

| PHENO-<br>TYPE | MEAN BODY WEIGHT (GRAMS) |          |     |          | MEAN BODY LENGTH (CM) |          |     |          | MEAN TAIL LENGTH (CM) |          |     |          |
|----------------|--------------------------|----------|-----|----------|-----------------------|----------|-----|----------|-----------------------|----------|-----|----------|
|                | NO.                      | ♂ ♂      | NO. | ♀ ♀      | NO.                   | ♂ ♂      | NO. | ♀ ♀      | NO.                   | ♂ ♂      | NO. | ♀ ♀      |
| b              | 48                       | 26.82    | 61  | 21.81    | 48                    | 9.51     | 61  | 9.13     | 48                    | 7.96     | 61  | 7.71     |
| B              | 72                       | 25.17    | 62  | 21.70    | 72                    | 9.18     | 62  | 9.10     | 72                    | 7.96     | 62  | 7.63     |
| Diff. Means    |                          | 1 65±.60 |     | .11±.55  |                       | .33±.09  |     | .03±.07  |                       | -.02±.09 |     | .08±.06  |
| se             | 57                       | 25.62    | 61  | 21.42    | 57                    | 9.41     | 61  | 9.11     | 57                    | 7.73     | 61  | 7.66     |
| SE             | 63                       | 26.19    | 62  | 22.08    | 63                    | 9.37     | 62  | 9.11     | 63                    | 8.02     | 62  | 7.68     |
| Diff. Means    |                          | -.57±.59 |     | -.66±.55 |                       | .04±.08  |     |          |                       | -.29±.10 |     | -.02±.08 |
| p              | 28                       | 26.23    | 46  | 22.56    | 28                    | 9.34     | 61  | 9.10     | 28                    | 7.92     | 61  | 7.62     |
| P              | 92                       | 25.81    | 77  | 21.29    | 92                    | 9.41     | 62  | 9.12     | 92                    | 7.99     | 62  | 7.68     |
| Diff. Means    |                          | .42±.61  |     | 1.27±.57 |                       | -.07±.07 |     | -.02±.06 |                       | -.07±.11 |     | -.06±.06 |

and specific factors influencing size. FELDMAN (unpublished) obtained data on some  $F_2$  and backcross generations which seemed to indicate that smaller body size was associated with piebald. Generally, waltzing mice are found to be smaller than their normal sibs.

The mice used were the Gates strain and the smaller Japanese strain described in the preceding cross.  $F_1$  mice were obtained by crossing ♀ Gates by ♂ Japanese. The  $F_1$  animals were backcrossed to the Japanese strains, using only ♀  $F_1$  by ♂ Japanese crosses. Some difficulty was encountered in breeding these animals. Thus a regrettably small number of backcross mice was obtained, and these were collected over the most part of a year.

Five size characters were studied. Adult body weight was taken on the 181st day. Mice were then chloroformed and body length and tail length measurements obtained. The tail was then cleared in KOH and glycerine and the number of caudal vertebrae counted. By using a vernier caliper the length of the 15th vertebra was measured to the nearest tenth millimeter.

Table 3 gives the mean adult measurements with the corresponding standard deviations as well as the differences of the means. In both sexes an almost constant feature is that black animals are largest, followed in decreasing order by black piebald, black waltzing, and black piebald waltzing animals. In body weight, among the females, waltzers are 6.4 percent lighter and animals homozygous for both recessives are 12.2 percent lighter than their normal black sisters.

In order to make the measurements comparable the total number of waltzers (*BvvSs* and *Bvvss*) is compared with all normal mice (*BVvSs* and *BVvss*), weighted equally. In like manner, all piebald mice (*BVvss* and *Bvvss*) are compared with all normal mice (*BVvSs* and *BvvSs*). Waltzing mice are smaller than their normal sibs in all characters studied, significantly so in body weight and length of caudal vertebrae.

TABLE 2

*Influence of particular genes on body size in the backcross to the  $F_1$  animals as indicated by a percentage increase or decrease (—) of the average.*

|                  | MALES          |                |                | FEMALES        |                |                |
|------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                  | BODY<br>WEIGHT | BODY<br>LENGTH | TAIL<br>LENGTH | BODY<br>WEIGHT | BODY<br>LENGTH | TAIL<br>LENGTH |
| Brown            | 6.30           | 3.51           | .27            | .51            | .44            | 1.04           |
| Dilute short-ear | -2.17          | .42            | -3.60          | -3.05          | 0              | -.27           |
| Pink-eye         | 1.60           | -.74           | -.89           | 5.87           | -.22           | -.79           |

Piebald mice are smaller than their unspotted sibs for all characters except tail length in males. Here the mean differences in some cases are almost negligible, but the same trend is evident.

If analysis of variance is used, the data may be better analyzed. In this manner the entire data are combined, and variance due to known causes determined. Known causes of variance in this cross are sex, the genes *s* and *v*. For combined data the mean square body weight of non-waltzing mice is 19.74 grams and for waltzers 17.25 grams. The mean square difference of  $2.49 \pm .64$  grams gives  $P = .01$ . Values of *P* are secured from Fisher's tables. The mean square difference between piebald and self mice is only  $.56 \pm .62$ . The value of *P* here is .30. The former is expected to be exceeded in random sampling from a homogeneous population only once in a hundred trials, the latter thirty times. Since the former value obtained is actually less than the one percent point such a value would not appear in random sampling even in one percent of the trials, and it is thus highly significant. The latter value  $P = .30$  cannot be considered statistically significant.

The mean square differences for body length and tail length in com-

TABLE 3  
Size measurements of different phenotypic classes from matings of ♀ *F*<sub>1</sub> by ♂ Japanese.

| PHENOTYPE              | NO. | SEX | MEAN BODY WEIGHT (GMS) | S.D.     | MEAN BODY LENGTH (CMS) | S.D.     | MEAN TAIL LENGTH (CMS) | S.D.     | MEAN NO. CAUDAL VERT. | S.D.     | MEAN 15TH VERT. LENGTH (MM) | S.D.    |
|------------------------|-----|-----|------------------------|----------|------------------------|----------|------------------------|----------|-----------------------|----------|-----------------------------|---------|
| Black                  | 24  | ♂♂  | 22.07±.58              | 1.93±.41 | 8.85±.33               | 1.05±.22 | 7.15±.35               | 1.18±.25 | 23.09±.20             | .67±.14  | 2.86±.06                    | .20±.04 |
|                        | 20  | ♀♀  | 17.99±.34              | 1.00±.20 | 8.35±.28               | 1.00±.20 | 6.98±.21               | .74±.15  | 23.37±.25             | .84±.18  | 2.88±.04                    | .16±.03 |
| Black Piebald          | 24  | ♂♂  | 21.22±.91              | 3.43±.46 | 8.70±.46               | 1.72±.32 | 7.19±.48               | 1.70±.32 | 23.27±.22             | .77±.15  | 2.80±.06                    | .24±.05 |
|                        | 22  | ♀♀  | 17.91±.73              | 2.58±.52 | 8.29±.30               | 1.05±.21 | 6.72±.41               | 1.45±.28 | 22.04±.42             | 1.36±.30 | 2.76±.10                    | .33±.07 |
| Black Waltzing         | 24  | ♂♂  | 18.77±1.34             | 3.53±.96 | 8.21±.65               | 1.73±.47 | 6.66±.46               | 1.2 ±.32 | 23.40±.44             | 1.00±.32 | 2.54±.06                    | .16±.05 |
|                        | 22  | ♀♀  | 16.84±.81              | 2.62±.58 | 8.04±.40               | 1.29±.28 | 6.34±.25               | .82±.18  | 22.50±.37             | 1.11±.26 | 2.69±.10                    | .31±.07 |
| Black Piebald Waltzing | 25  | ♂♂  | 17.66±1.11             | 3.33±.78 | 8.24±.22               | .65±.13  | 6.83±.49               | 1.48±.35 | 22.50±.37             | 1.00±.27 | 2.59±.11                    | .29±.08 |
|                        | 21  | ♀♀  | 15.79±.54              | 1.43±.38 | 7.96±.42               | 1.12±.30 | 6.37±.44               | 1.15±.31 | 22.60±.35             | .91±.25  | 2.56±.07                    | .20±.05 |
| Diff. means of V and v | ♂♂  |     | -3.45±1.34             |          | -54±.42                |          | -.42±.45               |          | -.30±.31              |          | -.26±.08                    |         |
|                        | ♀♀  |     | -1.54±.63              |          | -.32±.43               |          | -.50±.35               |          | -.30±.51              |          | -.19±.08                    |         |
| Diff. means of S and s | ♂♂  |     | -.96±.94               |          | -.09±.43               |          | .02±.45                |          | -.25±.27              |          | -.02±.07                    |         |
|                        | ♀♀  |     | -.30±.69               |          | -.04±.36               |          | -.06±.32               |          | -.45±.46              |          | -.11±.08                    |         |

paring both waltzing and piebald mice with their normal sibs are as follows: waltzers are smaller by  $.55 \pm .09$  centimeters. This gives  $P = .01$ . Piebald mice are smaller by only  $.03 \pm .09$  cms.  $P = .70$  which is not significant. In tail length, waltzers are smaller by  $.53 \pm .09$  cms where  $P = .01$ , which may be regarded as highly significant. As regards piebald there is no significant difference of the mean squares.

The question arises as to whether the mean differences obtained are representative of true genetic effects of the waltzing and piebald factors (or closely linked size factors). The above mean differences, especially those observed between waltzers and non-waltzers are relatively large. The approximate mean weight of waltzing mice is 17.5 grams as compared with a mean weight of 24.8 grams for the Gates strain. The Japanese animal is approximately 30 percent smaller. If it is assumed that the difference between the mean body weights is determined principally by genetic factors located on the chromosome bearing the waltzing gene, this one chromosome pair out of twenty accounts for nearly 30 percent of the difference in body weight. If such observed results are genetic in the sense of being determined by size factors in this chromosome, these must necessarily be classed as "major" size factors, as are possibly brown and dilution, but exerting a contrary effect to these.

It may be argued that the smaller general body size of the waltzers is due to the constant weaving motions of these mice. From periodic weighings, however, it was found that the logarithmic increase in weight for female waltzers over the two month period, four months to six months of age, is .02469 and for non-waltzers .02320. Waltzing males have an increase in body weight of .02410, whereas their non-waltzing brothers increase by only .00620. Early growth curves were not obtained, but it is evident from constant handling of the animals that waltzers are smaller from birth.

There is the possibility that the recessive genes waltzing and piebald have deleterious effects on the growth of the animal. However, brown and dilution, which are recessive genes, apparently exert a contrary effect on growth.

In a cross involving these two strains of mice GATES (1926) reported that an "association system" of chromosomes was formed such that certain combinations were found in excess of those expected in independent assortment. PAINTER (1927) gave cytological evidence showing that the bi-valents formed at the first maturation division in the  $F_1$  mice exhibited a peculiar behavior.

The mice used in this cross, although the same as used by GATES, were inbred to a greater degree. It is true that the doubly homozygous, piebald waltzing animals, are generally smaller than the single homozygotes, but from the data obtained it can be seen that there is no indication of

TABLE 4  
Size measurement of different phenotypic classes of backcross matings of ♀ *F*<sub>1</sub> by ♂ *P*incus.

| PHENOTYPE              | NO. | SEX | MEAN BODY       |            | S.D. | MEAN BODY       |            | S.D. | MEAN TAIL       |            | S.D. | MEAN NO.        |            | S.D. | MEAN LENGTH        |           | S.D. |
|------------------------|-----|-----|-----------------|------------|------|-----------------|------------|------|-----------------|------------|------|-----------------|------------|------|--------------------|-----------|------|
|                        |     |     | WEIGHT<br>(GMS) |            |      | LENGTH<br>(CMS) |            |      | LENGTH<br>(CMS) |            |      | CAUDAL<br>VERT. |            |      | 15TH VERT.<br>(MM) |           |      |
| Brown                  | 59  | ♂ ♂ | 29.38 ± .75     | 5.79 ± .53 |      | 9.67 ± .23      | 1.8 ± .17  |      | 8.66 ± .19      | 1.36 ± .13 |      | 24.91 ± .11     | .76 ± .08  |      | 2.91 ± .04         | .22 ± .03 |      |
|                        | 60  | ♀ ♀ | 24.59 ± .72     | 5.57 ± .51 |      | 9.24 ± .11      | .88 ± .08  |      | 8.31 ± .19      | 1.45 ± .14 |      | 24.87 ± .39     | 2.9 ± .27  |      | 2.88 ± .05         | .32 ± .03 |      |
| Brown Piebald          | 46  | ♂ ♂ | 28.33 ± .86     | 5.91 ± .62 |      | 9.58 ± .19      | 1.26 ± .13 |      | 8.49 ± .22      | 1.40 ± .15 |      | 24.73 ± .28     | 1.74 ± .20 |      | 2.92 ± .07         | .40 ± .05 |      |
|                        | 44  | ♀ ♀ | 24.23 ± .58     | 3.86 ± .41 |      | 9.33 ± .19      | 1.23 ± .16 |      | 8.39 ± .20      | 1.33 ± .14 |      | 24.97 ± .31     | 1.94 ± .22 |      | 2.83 ± .04         | .22 ± .03 |      |
| Black                  | 68  | ♂ ♂ | 27.55 ± .43     | 3.47 ± .28 |      | 9.52 ± .16      | 1.37 ± .12 |      | 8.55 ± .18      | 1.38 ± .13 |      | 24.49 ± .15     | 1.07 ± .10 |      | 2.86 ± .03         | .20 ± .02 |      |
|                        | 74  | ♀ ♀ | 23.41 ± .48     | 4.07 ± .33 |      | 9.12 ± .16      | 1.91 ± .16 |      | 8.14 ± .18      | 1.57 ± .13 |      | 24.43 ± .27     | 2.09 ± .19 |      | 2.78 ± .03         | .21 ± .02 |      |
| Black Piebald          | 46  | ♂ ♂ | 27.30 ± .58     | 3.93 ± .58 |      | 9.54 ± .18      | 1.24 ± .13 |      | 8.58 ± .21      | 1.43 ± .15 |      | 24.47 ± .11     | .70 ± .08  |      | 2.59 ± .04         | .24 ± .03 |      |
|                        | 55  | ♀ ♀ | 23.96 ± .59     | 4.37 ± .41 |      | 9.01 ± .15      | 1.13 ± .11 |      | 8.18 ± .18      | 1.46 ± .14 |      | 24.64 ± .22     | 1.6 ± .15  |      | 2.75 ± .03         | .21 ± .02 |      |
| Diff. means of B and b | 219 | ♂ ♂ | 1.47 ± .66      |            |      | .07 ± .19       |            |      | .04 ± .18       |            |      | .35 ± .16       |            |      | .18 ± .13          |           |      |
|                        | 233 | ♀ ♀ | .79 ± .60       |            |      | .21 ± .17       |            |      | .19 ± .19       |            |      | .40 ± .3c       |            |      | .10 ± .09          |           |      |
| Diff. means of Sand s  | 219 | ♂ ♂ | -.58 ± .68      |            |      | -.03 ± .19      |            |      | -.07 ± .18      |            |      | -.10 ± .16      |            |      | -.14 ± .15         |           |      |
|                        | 233 | ♀ ♀ | .14 ± .58       |            |      | -.02 ± .18      |            |      | .14 ± .19       |            |      | .15 ± .28       |            |      | -.05 ± .08         |           |      |

such an "association system." The backcross segregates recorded by CASTLE, GATES and REED (1936), resulting from a cross of parental strains similar to those employed by GATES, showed no significant deviations from expectation.

### CROSS 3

#### The ♀ F<sub>1</sub> by ♂ Pincus Backcross

This backcross was made in the hope of securing further data on two questions of general theoretical interest. First, is the question of whether the correlation of larger body size and brown coat color observed by GREEN (1931) and by FELDMAN (1935) and a similar association involving both brown and dilution (CASTLE, GATES, REED and LAW, 1936) may be attributed to the influences of size factors located on the chromosomes bearing these markers, or whether these phenomena result from the physiological action of the color genes *per se* upon growth processes.

A second point of interest concerns the chromosome bearing piebald (*s*). In the previous ♀ F<sub>1</sub> by ♂ Japanese cross there was some indication of a correlation between smaller general body size and piebald.

The Pincus strain has been inbred, brother by sister, for more than twenty generations. Due to the paucity of this stock, adult measurements are from small numbers of mice. From the growth curves of MARSHAK (1936) adult weight obtained on the 181st day is nearly the same for both sexes, 23 grams. Mean body length for both sexes combined was found to be 10.22 centimeters, and mean tail length 7.92 centimeters. Four chromosomes were marked by the following genes, *C*, *a*, *b*, and *s*. Thus genotypically they are *CCaabbss*.

The F<sub>1</sub> animals used were those described in the first cross, from ♀ Gates by ♂ Japanese. The chromosome marked by *b* is derived from the larger Gates strain and the chromosome marked by *s* from the smaller Japanese waltzing strain. The backcross was made in only one direction, using females of the F<sub>1</sub> stock and males of the inbred Pincus line. Backcross mice segregated into four classes, brown, brown piebald, black, and black piebald.

A total of 452 animals constitutes the backcross population. Size measurements were obtained over a relatively short period of time, six months. Animals were weighed every two months, and adult body weight was obtained on the 181st day at which time all other measurements were secured.

By reference to table 4 it can be seen that brown (*b*) animals are larger than black (*B*) mice for all size measurements. If all the brown males, *bbSs* and *bbss* are compared with all black males, *BbSs* and *Bbss*, weighted equally, the differences of the means are all in favor of the brown mice.

Only the differences in body weight, number of caudal vertebrae, and length of vertebra among the males, and length of 15th vertebra in females can be considered of statistical significance, but all are in the same direction.

The results as regards the effects on general body size of piebald (*s*) or closely linked size factors, are not clear-cut. There was some indication from the second cross, where *s* was introduced by the smaller Japanese parent, as well as from data of FELDMAN that piebald mice were generally smaller than their normal sibs. If we compare all piebald mice (*bbss* and *Bbss*) with all heterozygous non-piebald mice (*bbSs* and *BbSs*), it is seen that among the males piebald mice are smaller for all size measurements obtained. These mean differences in some cases are almost negligible but all in the same direction. In the female backcross population piebald mice are smaller in only body length and length of caudal vertebra. It is expected that males should provide the most reliable data.

Upon the assumption that size factors, possibly inhibitory in effect, have become associated with the piebald factor in the smaller Japanese race, we might expect these to be absent in the larger Pincus strain, or through selection this piebald marked chromosome may have accumulated size factors favorable to or stimulating growth. If such is the case, the piebald backcross segregates in this particular cross should not necessarily show smaller size, but may possibly be larger than their non-piebald sibs. If, on the other hand, the piebald factor itself is physiologically influencing growth, this factor should produce the same effect no matter from what strain it is derived. From the data obtained it can be seen that there is a slight indication that the piebald gene, like the dilution and short-ear genes, influences body size.

#### CROSS 4

The ♀  $F_1$  wild (Little/wild) by ♂ Little backcross  
and its reciprocal, the ♀ Little by  
♂  $F_1$  wild cross

In the crosses thus far studied three marked chromosomes have given indication of bearing genes having a favorable influence on the growth of mice obtained in the backcross generations. Brown mice are larger than their dominant black sibs. Dilution mice are likewise larger than their dominant intense sibs. Although the dilution effect was somewhat less pronounced in body measurements, as compared with the brown factor or factors, a more definite favorable effect was noted on tail measurements, particularly length of tail and number of tail rings and number of caudal vertebrae. This effect must necessarily be spoken of, as apparent, since it is not known whether the size measurements used are comparable. The

chromosome carrying agouti has not given results that may be considered as conclusive as the other two. GREEN's earlier work (1931) showed that non-agouti mice were slightly larger than agoutis. In a later publication (1931b), using the same inbred strains of mice he reports that the agouti mice (agouti coming from the smaller parent) tend to exceed non-agoutis in size. In a cross already reported (CASTLE et al 1936 II) we could find no consistent influence either on increase or decrease in size. Non-agouti females were larger than males by all three criteria used; body weight, body length and tail length. But among the males a contrary relation was found. Further studies on three additional size characters, tail ring number, number of caudal vertebrae and length of 15th caudal vertebra showed that the mice homozygous for non-agouti (which came into the cross from the larger *musculus* race) had a significantly greater tail ring number in both backcrosses.

The parental strains used in this cross were the dba strain of Little and a wild (DBA) inbred strain of mice. Unfortunately the latter strain is not highly inbred. The wild strain is slightly larger in body measurements than the Little strain. The measurements obtained from the pure Little strain were body weight 23.54 grams, body length 9.70 cms, tail length 8.15 cms. For the wild animals these were body weight 24.50 grams, body length 9.72 cms and tail length 8.23 cms. These measurements are for males and females combined. The  $F_1$  animals show heterosis effects, being considerably larger than either of the two parental strains. The measurements obtained from these  $F_1$  animals (males and females) were 29.40 grams for body weight, 10.41 cms for body length and 8.91 cms for tail length.

This cross involves the same genes as the crosses made by GREEN (1931) and by CASTLE, GATES, REED and LAW (1936). In those crosses the three chromosomes marked by *D*, *B*, and *A* come from the smaller *bat-trianus* strain. In the present cross the chromosomes marked by the three independent genes come in from the slightly larger wild *musculus* parent. The backcross populations which were made reciprocally comprise 673 animals. One additional size character has been studied in these crosses, namely, tail ring number. The importance of this character in quantitative studies was first reported by FORTUYN (1930), although taxonomists have long recognized its value.

In both backcrosses brown animals are larger than the black sibs for all six measurements obtained (tables 5 and 6). This is more pronounced in adult body weight (181st day), body length and tail length. If the two backcross populations are combined, males and females, and the variance caused by reciprocal crossing, sex and color (in this case, brown) is calculated, it is seen by reference to table 7 that brown animals are



TABLE 5  
Comparative size measurements of different phenotypic classes of backcross mice from matings of ♀ Little (dba) by ♂ F<sub>1</sub> wild.

| PHENO-<br>TYPE | MEAN BODY WEIGHT (GMS) |             |     | MEAN BODY LENGTH (CM) |             |             | MEAN TAIL LENGTH (CM) |             |             | MEAN TAIL RING NO. |             |             | MEAN NO. OF CAUDAL<br>VERTEBRAE |             |    | MEAN LENGTH OF 15TH<br>VERT. (MM) |   |   |
|----------------|------------------------|-------------|-----|-----------------------|-------------|-------------|-----------------------|-------------|-------------|--------------------|-------------|-------------|---------------------------------|-------------|----|-----------------------------------|---|---|
|                | NO.                    | ♂♂          | ♀   | ♂♂                    | ♀           | ♂♂          | ♂♂                    | ♀           | ♂♂          | ♀                  | ♂♂          | ♀           | ♂♂                              | ♀           | ♂♂ | ♂♂                                | ♀ | ♀ |
| b              | 87                     | 28.67       | 102 | 23.89                 | 10.39       | 10.01       | 8.60                  | 8.36        | 147.4       | 148.9              | 26.28       | 26.24       | 3.51                            | 3.37        |    |                                   |   |   |
| B              | 82                     | 28.17       | 98  | 23.41                 | 10.25       | 9.92        | 8.46                  | 8.21        | 146.6       | 148.3              | 25.85       | 25.96       | 3.44                            | 3.34        |    |                                   |   |   |
| Diff. Means    |                        | + .50 ± .56 |     | + .48 ± .15           | + .14 ± .15 | ± .09 ± .08 | + .14 ± .07           | + .15 ± .6  | + 0.8 ± .71 | ± 0.6 ± .63        | + .43 ± .13 | + .28 ± .24 | + .07 ± .04                     | + .03 ± .06 |    |                                   |   |   |
| d              | 77                     | 28.15       | 108 | 23.78                 | 10.31       | 10.03       | 8.55                  | 8.31        | 148.2       | 150.4              | 26.28       | 26.19       | 3.53                            | 3.36        |    |                                   |   |   |
| D              | 92                     | 28.69       | 92  | 23.70                 | 10.35       | 9.92        | 8.51                  | 8.27        | 146.1       | 148.4              | 25.96       | 25.99       | 3.45                            | 3.34        |    |                                   |   |   |
| Diff. Means    |                        | - .54 ± .51 |     | - .01 ± .38           | - .04 ± .15 | + .11 ± .05 | + .04 ± .07           | + 2.1 ± .70 | + .04 ± .06 | + 2.0 ± .69        | + .32 ± .12 | + .20 ± .19 | + .08 ± .04                     | + .02 ± .04 |    |                                   |   |   |
| a              | 77                     | 27.80       | 95  | 23.68                 | 10.26       | 9.94        | 8.53                  | 8.22        | 145.2       | 147.4              | 26.16       | 26.95       | 3.49                            | 3.32        |    |                                   |   |   |
| A              | 92                     | 28.84       | 105 | 23.80                 | 10.35       | 9.99        | 8.55                  | 8.32        | 148.1       | 149.1              | 26.06       | 26.11       | 3.46                            | 3.39        |    |                                   |   |   |
| Diff. Means    |                        | + .98 ± .49 |     | + .12 ± .46           | + .07 ± .15 | + .05 ± .07 | + .02 ± .06           | + .10 ± .06 | + 2.9 ± .69 | + 1.6 ± .68        | - .10 ± .11 | + .06 ± .19 | - .03 ± .05                     | + .07 ± .06 |    |                                   |   |   |

TABLE 6  
Comparative size measurements of different phenotypic classes of backcross mice from matings of ♀ F<sub>1</sub> wild by ♂ Little (dba).

| PHENOTYPE   | MEAN BODY WEIGHT (GMS) |             |    | MEAN BODY LENGTH (CM) |             |             | MEAN TAIL LENGTH (CM) |             |             | MEAN TAIL RING NO. |             |             | MEAN CAUDAL VERTEBRAE<br>NO. |             |    | MEAN LENGTH OF 15TH<br>VERTEBRAE (MM) |   |   |
|-------------|------------------------|-------------|----|-----------------------|-------------|-------------|-----------------------|-------------|-------------|--------------------|-------------|-------------|------------------------------|-------------|----|---------------------------------------|---|---|
|             | NO.                    | ♂♂          | ♀  | ♂♂                    | ♀           | ♂♂          | ♂♂                    | ♀           | ♂♂          | ♀                  | ♂♂          | ♀           | ♂♂                           | ♀           | ♂♂ | ♂♂                                    | ♀ | ♀ |
| b           | 56                     | 28.26       | 74 | 24.14                 | 10.36       | 10.12       | 8.64                  | 8.43        | 150.1       | 152.8              | 26.40       | 26.20       | 3.45                         | 3.39        |    |                                       |   |   |
| B           | 86                     | 27.29       | 87 | 23.47                 | 10.26       | 9.97        | 8.47                  | 8.19        | 148.2       | 152.6              | 25.88       | 25.89       | 3.39                         | 3.31        |    |                                       |   |   |
| Diff. Means |                        | + .97 ± .58 |    | + 67 ± .47            | + .10 ± .06 | + .15 ± .06 | + .17 ± .12           | + .24 ± .12 | + 1.9 ± .94 | + 0.2 ± .82        | + .32 ± .16 | + .40 ± .16 | + .06 ± .06                  | + .08 ± .05 |    |                                       |   |   |
| d           | 67                     | 27.81       | 77 | 23.52                 | 10.32       | 10.06       | 8.61                  | 8.32        | 150.6       | 156.3              | 26.34       | 26.10       | 3.40                         | 3.32        |    |                                       |   |   |
| D           | 75                     | 27.77       | 84 | 23.63                 | 10.28       | 10.01       | 8.53                  | 8.26        | 148.4       | 149.1              | 25.93       | 25.87       | 3.42                         | 3.46        |    |                                       |   |   |
| Diff. Means |                        | + .04 ± .62 |    | - .09 ± .49           | + .04 ± .06 | + .05 ± .06 | + .08 ± .07           | + .06 ± .08 | + 2.2 ± .85 | + 7.2 ± .67        | + .41 ± .18 | + .32 ± .16 | + .02 ± .07                  | - .14 ± .06 |    |                                       |   |   |
| a           | 65                     | 27.52       | 71 | 23.89                 | 10.29       | 10.02       | 8.58                  | 8.35        | 150.1       | 152.3              | 26.23       | 26.18       | 3.41                         | 3.39        |    |                                       |   |   |
| A           | 77                     | 27.59       | 90 | 23.39                 | 10.32       | 10.04       | 8.54                  | 8.27        | 148.9       | 152.9              | 26.05       | 25.97       | 3.42                         | 3.31        |    |                                       |   |   |
| Diff. Means |                        | + .07 ± .60 |    | - .50 ± .40           | + .03 ± .06 | + .02 ± .06 | - .04 ± .08           | - .08 ± .12 | - 1.2 ± .85 | - 0.6 ± .73        | - .18 ± .18 | - .21 ± .14 | + .01 ± .06                  | - .08 ± .05 |    |                                       |   |   |

significantly larger than their black sibs in all size measurements with the exception of tail ring number.

Dilute mice are not consistently heavier or longer-bodied than intense

TABLE 7

*Analysis of variance of backcross progeny from matings of ♀ F<sub>1</sub> wild by ♂ Little and ♀ Little by ♂ F<sub>1</sub> wild crosses.*

| MEASUREMENT                                | VARIANCE DUE TO    | DEGREES<br>OF<br>FREEDOM | SUM OF<br>SQUARES | S. D. | MEAN SQUARE<br>DIFFERENCE<br>AND P |
|--|--------------------|--------------------------|-------------------|-------|------------------------------------|
| Body weight (grams)                        | Total              | 672                      | 12053.71          | 3.37  | .49 ± .23<br><br>P = .05           |
|  | reciprocal crosses | 1                        | 16.30             |       |                                    |
|  | sex                | 1                        | 4379.38           |       |                                    |
|  | color (brown)      | 1                        | 38.80             |       |                                    |
|  | residual           | 669                      | 7629.25           |       |                                    |
| Body length (centi-<br>meters)             | Total              | 587                      | 366.15            | .78   | .20 ± .06<br><br>P < .01           |
|  | reciprocal crosses | 1                        | 0.14              |       |                                    |
|  | sex                | 1                        | 9.18              |       |                                    |
|  | color (brown)      | 1                        | 5.38              |       |                                    |
|  | residual           | 584                      | 351.45            |       |                                    |
| Tail length (centi-<br>meters)             | Total              | 517                      | 177.2             | .57   | .18 ± .05<br><br>P < .01           |
|  | reciprocal crosses | 1                        | 0.1               |       |                                    |
|  | sex                | 1                        | 7.9               |       |                                    |
|  | color (brown)      | 1                        | 4.2               |       |                                    |
|  | residual           | 514                      | 165.0             |       |                                    |
| Tail rings                                 | Total              | 504                      | 9215.0            | 4.24  | .40 ± .37<br><br>P = .3            |
|  | reciprocal crosses | 1                        | 133.6             |       |                                    |
|  | sex                | 1                        | 77.3              |       |                                    |
|  | color (brown)      | 1                        | 1.5               |       |                                    |
|  | residual           | 501                      | 9002.6            |       |                                    |
| No. caudal vertebrae                       | Total              | 426                      | 1697.4            | 1.99  | .43 ± .19<br><br>P < .05           |
|  | reciprocal crosses | 1                        | 0                 |       |                                    |
|  | sex                | 1                        | 0.1               |       |                                    |
|  | color (brown)      | 1                        | 15.0              |       |                                    |
|  | residual           | 423                      | 1682.3            |       |                                    |
| Length of 15th verte-<br>bra (millimeters) | Total              | 424                      | 46.7              | .33   | .08 ± .03<br><br>P < .01           |
|  | reciprocal crosses | 1                        | 0                 |       |                                    |
|  | sex                | 1                        | 1.1               |       |                                    |
|  | color (brown)      | 1                        | .5                |       |                                    |
|  | residual           | 421                      | 45.1              |       |                                    |

mice. As in other crosses the effect of the dilution gene is apparently greatest in the tail region, where significant differences in the number of tail rings and number of caudal vertebrae are in favor of the dilute segregates.

Agouti mice are not consistently heavier than non-agoutis. In the ♀

Little by ♂ F<sub>1</sub> wild cross, both males and females are heavier. The other mean differences in a positive direction are almost negligible. Females in the reciprocal ♀ F<sub>1</sub> wild by ♂ Little backcross show a contrary relationship. All agouti mice are longer-bodied, but again the mean differences are too small to be conclusive. CASTLE (1934), in attempting to locate genes influencing general body size in rabbits, and LIVESAY (1930) working with rats, could find no association of the agouti gene with genes favorable to general body growth, although in both reports there was some indication of their existence.

TABLE 8

*Analysis of variance of backcross progeny from matings of ♀ F<sub>1</sub> wild by ♂ Little and of ♀ Little by ♂ F<sub>1</sub> wild crosses.*

| MEASUREMENT                   | VARIANCE DUE TO    | DEGREES<br>OF FREEDOM | SUM OF<br>SQUARES | S.D. | MEAN SQ.<br>DIFF. AND P |
|-------------------------------|--------------------|-----------------------|-------------------|------|-------------------------|
| Tail length                   | Total              | 519                   | 111.4             | .45  | .11 ± .04               |
|                               | reciprocal crosses | 1                     | .04               |      |                         |
|                               | sex                | 1                     | 8.38              |      |                         |
|                               | color (dilution)   | 1                     | .24               |      |                         |
|                               | residual           | 516                   | 102.74            |      |                         |
| Number of caudal<br>vertebrae | Total              | 426                   | 1902.0            | 2.11 | .51 ± .25               |
|                               | reciprocal crosses | 1                     | .0                |      |                         |
|                               | sex                | 1                     | .0                |      |                         |
|                               | color (dilution)   | 1                     | 7.0               |      |                         |
|                               | residual           | 423                   | 1895.0            |      |                         |
| Number of tail rings          | Total              | 503                   | 8476.1            | 4.1  | .99 ± .37               |
|                               | reciprocal crosses | 1                     | 155.77            |      |                         |
|                               | sex                | 1                     | 65.37             |      |                         |
|                               | color (non-agouti) | 1                     | 22.72             |      |                         |
|                               | residual           | 500                   | 8232.14           |      |                         |

However, agouti mice have a greater number of tail rings if all data are combined. Although the cross ♀ F<sub>1</sub> wild by ♂ Little shows a very slight trend in the opposite direction, the significant differences of the reciprocal cross, which constituted almost twice the number of animals, more than offsets this contrary effect. (See table 8.)

The data obtained from this series of crosses indicate that the brown gene acts in a manner similar to the dilution gene in exerting a favorable influence on growth. Two separate lines of evidence support the physiological explanation of this gene *per se*. In all crosses made, regardless of the strains used, brown backcross mice are larger than their black sibs. The chromosome carrying the brown gene under consideration was introduced by three different inbred strains, the Little strain, the smaller

Gates race, and the Pincus strain. Other crosses made by FELDMAN (1935) and GREEN (1935a) in which still different inbred strains of brown mice were used gave similar results. Green intended to show that the brown gene was not always associated with genes favorably influencing growth. His data show, however, that the brown backcross segregates are indeed larger than their heterozygous black sibs. Although the mean differences are not of statistical significance, probably because of the small number of mice obtained, these results on the contrary add support to the physiological interpretation.

Further evidence supporting this assumption comes from two crosses representing coupling and repulsion phases. In a backcross involving the small *bactrianus* strain of mice and the larger Little strain the chromosome carrying the brown gene came into the cross from the much larger parent CASTLE et al 1936 II). The brown segregates were found to be significantly larger than the black sibs. In this backcross the same Little strain contributed the chromosome marked by the brown gene, although this strain was slightly smaller than the wild strain used as the other parental group. Brown backcross segregates were again larger as determined by a series of five measurements.

This evidence does not eliminate the possibility of an extremely close linkage between the brown gene and size genes located on the same chromosome.

#### CROSS 5

The ♀ Snell by ♂ F<sub>1</sub> Snell (Snell/wild) backcross

Data from backcross matings of the GATES and F<sub>1</sub> strains indicated that the short-ear gene decreased general body size in the presence of dilution, even though the dilution gene presumably has a favorable effect on growth when separated from the short-ear gene.

It was possible to obtain an inbred strain of mice homozygous for *se* and for *D*. This strain, referred to as the SNELL strain, was homozygous for extreme dilution (*c<sup>h</sup>*), an albino allele, and for *se*. These animals weighed approximately 25 grams and were slightly larger than the inbred wild strain already discussed in a previous cross. Snell strain females were crossed with wild strain males. The F<sub>1</sub> animals were somewhat larger than either parental race. For 15 animals (males and females combined) the mean body weight was 26.28 grams, body length 9.81 cms, and tail length 8.13 cms. The backcross was made using the Snell race as mothers. Backcross offspring fall into four classes: *c<sup>h</sup> c<sup>h</sup> se se*, *c<sup>h</sup> c<sup>h</sup> se se*, *Cc<sup>h</sup> SE se*, and *Cc<sup>h</sup> se se*. The intense animals were agoutis and non-agoutis combined. Among the extreme dilute animals the agouti pattern could not be distinguished.

Growth curves obtained from 18 litters of mice show that up until the second week of age both short-ear and normal-ear mice are growing at the same rate. After the 12th day in females and the 17th day in males normal-ear mice are constantly heavier and this difference in weight gradually widens (see fig. 1 and 2). At the five-week period on the integral curve the short-ear females are smaller than their normal sisters by  $1.10 \pm .97$

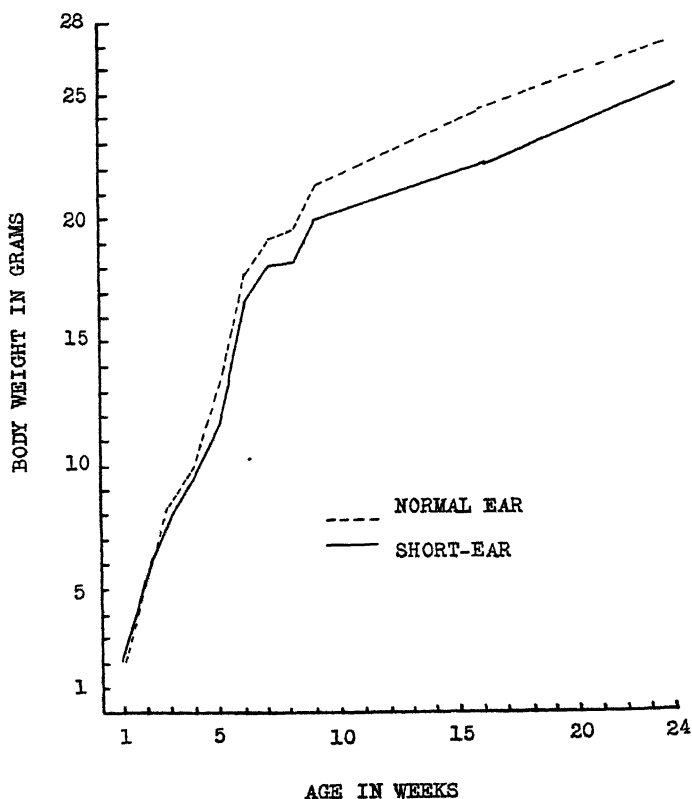


FIGURE 1.—Integral growth curves for female backcross segregates from matings of ♀ Snell by ♂  $F_1$  mice.

grams and short-ear males are smaller by  $1.68 \pm 1.20$  grams. At the twelve-week point on the curve the mean difference in weight for males is  $2.16 \pm 1.18$  grams and for females  $2.91 \pm 1.55$  grams.

Three measurements taken were adult body weight on the 181st day, body length, and tail length. By reference to table 9 it is seen that the short-ear segregates in both male and female populations are lighter in body weight. The decrease in weight expressed as a percentage decrease of the average is 6.4 percent for males and 10.5 percent for females. The mean differences for body length are less pronounced, but in the same direction. Likewise, the tail is shorter among the short-ear progeny.

The trend of the differences of the means for extreme dilute mice as compared with their colored sibs is not consistent.

If all backcross mice, male and female, short-ear and normal ear, are combined, the variance due to sex, the short-ear factor, and experimental error determined, a comparison of the mean squares should test the significance of differences between the short-ear and normal-ear groups. The values of *P* obtained for all three size criteria used are below the one percent point (table 10).

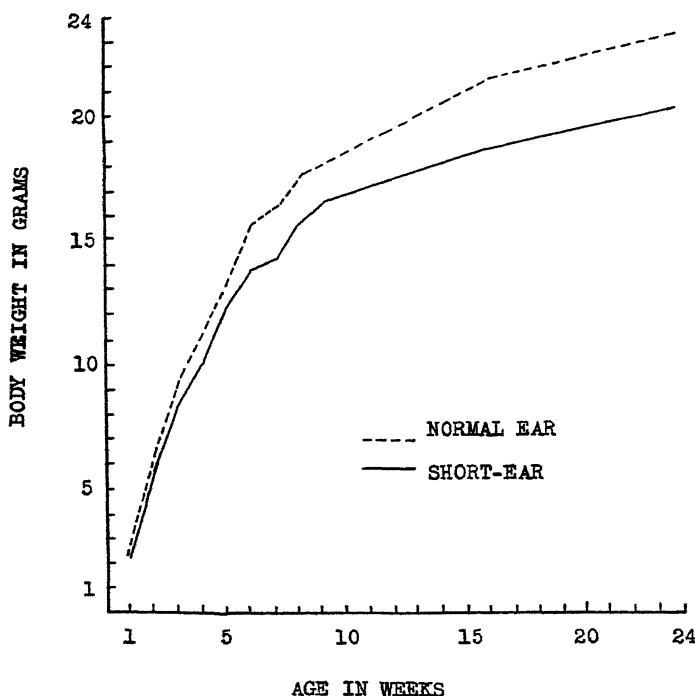


FIGURE 2.—Integral growth curves for male backcross segregates from matings of ♀ Snell by ♂  $F_1$  mice.

The small amount of data secured from Cross 5 does not permit a quantitative estimate of the influence of the short-ear gene. It does indicate, however, that this gene produces an effect 3 or 4 times as great in decreasing general body size than when it is closely linked with the dilution gene.

In both backcrosses involving short-ear mice the marked chromosome was introduced by the larger parent strain, from the Gates strain in Cross 1 and from the Snell strain in Cross 5. It is difficult to conceive of the observed phenomenon resulting from linkage of genes retarding growth with the short-ear gene. Rather, the results from these crosses add support to the physiological explanation given previously for the short-ear gene.

The question arises as to whether we are dealing with a set-up which

TABLE 9

*Comparative size measurements of different phenotypic classes of backcross mice from matings of ♀ Snell (c<sup>h</sup>se) by ♂ F<sub>1</sub> Snell.*

| PHENO-<br>TYPE | NO. | SEX | MEAN BODY<br>WEIGHT (GMS) | S.D.       | MEAN BODY<br>LENGTH (CMS) | S.D.       | MEAN TAIL<br>LENGTH (CMS) | S.D.       |
|----------------|-----|-----|---------------------------|------------|---------------------------|------------|---------------------------|------------|
| se             | 20  | ♂ ♂ | 25.71 ± .73               | 3.32 ± .53 | 9.95 ± .18                | .80 ± .12  | 7.96 ± .41                | 1.88 ± .29 |
| SE             | 21  |     | 27.39 ± .92               | 4.25 ± .65 | 10.06 ± .25               | 1.17 ± .18 | 8.18 ± .40                | 1.87 ± .28 |
| Diff. Means    |     |     | -1.68 ± 1.17              |            | -.11 ± .38                |            | -.22 ± .56                |            |
| c <sup>h</sup> | 20  | ♂ ♂ | 26.44 ± .86               | 3.87 ± .61 | 10.03 ± .24               | 1.09 ± .17 | 8.13 ± .44                | 1.98 ± .32 |
| C              | 21  |     | 26.70 ± .84               | 3.89 ± .59 | 9.99 ± .21                | .97 ± .15  | 8.01 ± .39                | 1.80 ± .27 |
| Diff. Means    |     |     | -.26 ± 1.12               |            | .04 ± .32                 |            | .12 ± .58                 |            |
| se             | 21  | ♀ ♀ | 20.23 ± .61               | 2.81 ± .43 | 9.33 ± .28                | 1.29 ± .19 | 7.49 ± .44                | 1.99 ± .30 |
| SE             | 21  |     | 23.22 ± .62               | 2.86 ± .44 | 9.69 ± .29                | 1.31 ± .20 | 8.13 ± .32                | 1.48 ± .23 |
| Diff. Means    |     |     | -2.29 ± .86               |            | -.36 ± .39                |            | -.64 ± .54                |            |
| c <sup>h</sup> | 20  | ♀ ♀ | 20.58 ± .70               | 3.18 ± .50 | 9.39 ± .27                | 1.20 ± .19 | 7.72 ± .45                | 2.05 ± .32 |
| C              | 22  |     | 22.28 ± .54               | 2.55 ± .38 | 9.63 ± .25                | 1.28 ± .19 | 7.92 ± .36                | 1.71 ± .25 |
| Diff. Means    |     |     | -1.70 ± .89               |            | -.24 ± .37                |            | -.20 ± .58                |            |

will give results easily interpreted concerning size inheritance. The short-ear gene, along with another tested gene, waltzing, may be deleterious in effect and possibly should not come under the classification of size genes.

Other effects are known to be produced by the short-ear gene *per se*.

TABLE 10

*Analysis of variance of backcross progeny from matings of ♀ Snell by ♂ F<sub>1</sub> Snell.*

| MEASUREMENT                    | VARIANCE<br>DUE TO | DEGREES OF<br>FREEDOM | SUM OF<br>SQUARES | S.D. | MEAN SQ. DIFF.<br>AND P |
|--------------------------------|--------------------|-----------------------|-------------------|------|-------------------------|
| Body weight (grams)            | Total              | 77                    | 1429.9            | 3.37 | 2.44 ± .76              |
|                                | sex                | 1                     | 464.9             |      |                         |
|                                | short-ear          | 1                     | 114.7             |      |                         |
|                                | residual           | 75                    | 850.3             |      |                         |
| Body length (centi-<br>meters) | Total              | 76                    | 16.0              | .37  | .52 ± .09               |
|                                | sex                | 1                     | 4.9               |      |                         |
|                                | short-ear          | 1                     | 1.2               |      |                         |
|                                | residual           | 74                    | 9.9               |      |                         |
| Tail length (centi-<br>meters) | Total              | 75                    | 29.4              | .58  | .44 ± .13               |
|                                | sex                | 1                     | 1.3               |      |                         |
|                                | short-ear          | 1                     | 3.5               |      |                         |
|                                | residual           | 73                    | 24.6              |      |                         |

GATES (1926) remarked that it produced morphological variations of the head and skull, and also pointed out that general body size seemed to be affected. SNELL (1931) showed that kinky tail was an effect of this gene. A morphological study showed that there was a proportionate increase in width of brain case, and a reduction in height of the rostrum in short-eared mice as compared with normal sibs. The writer (unpublished) has also found that backcross mice homozygous for short-ear are much more susceptible to *Salmonella aertrycke* than are their normal sibs.

If the short-ear gene may be spoken of as a size gene, it is a major size gene, as are brown and dilution, but produces a contrary effect. There are two reasons for assuming this. First, the observed mean differences between the short-ear and normal-ear segregates are great, whereas the parent strain differed only slightly in body size. Second, the effect was evident from a comparatively small backcross population.

#### THE SIZE CHARACTER, TAIL RING NUMBER

In 1931 FORTUYN crossed two inbred strains of mice differing greatly in tail ring number. These were the C58 strain with a low tail ring number and the Stoli strain having a relatively high number. The  $F_1$  was exactly intermediate between the parental groups, but backcross mice showed practically the same variability as the parental groups. It was suggested that linkage of factors influencing tail ring number with color factors might account for this. At that time no particular attention was paid to the different combinations of color factors obtained in the backcross.

This size character was included in the crosses made for various reasons. It was noted in earlier studies (CASTLE et al, 1936 I and II) that there was an apparent association between dilution and tail length. This seemed a good character for study since there was found a relatively high correlation between tail length and tail ring number ( $r = .39 \pm .06$ ). Furthermore, FORTUYN (1934) had reported some interesting results regarding the inheritance of this character. He reported that the inheritance of tail ring number behaved much like a case of segregation for a single pair of autosomal factors. Later FORTUYN (1936) reported that there were modifying genes in the Y chromosome, since in segregation of the basal autosomal factors dominance of the high tail ring number was incomplete, and segregation in the  $F_2$  was less pronounced than in typical cases. This suggestion was also adduced from the fact that in reciprocal crosses a significant difference was found between the male backcross populations.

Backcross mice of the following crosses were used for a study of tail ring number: 1) ♀  $F_1$  by ♂ Little (dba) and its reciprocal, 2) ♀ Gates by ♂  $F_1$ , and its reciprocal, 3) ♀  $F_1$  *bactriamus* by ♂ Little and reciprocal, and 4) the ♀  $F_1$  wild by ♂ Little and its reciprocal. Previous studies on



Cross 1 were reported by CASTLE, GATES and REED (1936) and for Cross 3 by CASTLE, GATES, REED and LAW (1936). The  $F_1$  animals from Crosses 1, 2 and 4 were obtained by crosses of ♀ Gates by ♂ Japanese. The  $F_1$

TABLE II

*Influence of the dilution gene (d) on the character tail ring number in various size crosses.*

| CROSS                                 | SEX | NO. | PHENOTYPE            | NO. TAIL RINGS | DIFF. MEANS | % INFLUENCE OF DILUTION MEASURED BY INCREASE OF AVERAGE | WEIGHTED MEAN |
|---------------------------------------|-----|-----|----------------------|----------------|-------------|---|---------------|
| 1) ♀ F <sub>1</sub> by ♂ Little       | ♀ ♀ | 325 | <i>D</i>             | 151.6 ± 1.5    | 5.8 ± 1.9   | 3.82  | 2.69          |
|                                       | ♀ ♀ | 345 | <i>d</i>             | 157.4 ± 1.3    |             |   |               |
|                                       | ♂ ♂ | 281 | <i>D</i>             | 154.7 ± 1.0    | 1.7 ± 1.5   | 1.09  |               |
|                                       | ♂ ♂ | 276 | <i>d</i>             | 156.4 ± 1.1    |             |   |               |
| ♀ Little by ♂ F <sub>1</sub>          | ♀ ♀ | 46  | <i>D</i>             | 146.6 ± 1.4    | 3.2 ± 1.9   | 2.19  | 2.72          |
|                                       | ♀ ♀ | 41  | <i>d</i>             | 149.8 ± 1.4    |             |   |               |
|                                       | ♂ ♂ | 26  | <i>D</i>             | 146.4 ± 1.6    | 4.9 ± 2.3   | 3.36  |               |
|                                       | ♂ ♂ | 36  | <i>d</i>             | 151.3 ± 1.7    |             |   |               |
| 2) ♀ F <sub>1</sub> by ♂ Gates        | ♀ ♀ | 418 | <i>D+SE</i> (linked) | 93.1 ± .53     | 1.2 ± .74   | 1.90  | 1.29          |
|                                       | ♀ ♀ | 397 | <i>d+se</i>          | 94.3 ± .52     |             |   |               |
|                                       | ♂ ♂ | 423 | <i>D+SE</i>          | 93.9 ± .63     | 1.8 ± .84   | 1.25  |               |
|                                       | ♂ ♂ | 396 | <i>d+se</i>          | 95.7 ± .56     |             |   |               |
| 3) ♀ F <sub>1</sub> bact. by ♂ Little | ♀ ♀ | 151 | <i>D</i>             | 95.6 ± .44     | 3.3 ± .56   | 3.43  | 3.33          |
|                                       | ♀ ♀ | 142 | <i>d</i>             | 98.9 ± .35     |             |   |               |
|                                       | ♂ ♂ | 144 | <i>D</i>             | 93.4 ± .48     | 3.0 ± .64   | 3.23  |               |
|                                       | ♂ ♂ | 135 | <i>d</i>             | 96.4 ± .43     |             |   |               |
| ♀ Little by ♂ F <sub>1</sub> bact.    | ♀ ♀ | 63  | <i>D</i>             | 109.0 ± .64    | 1.3 ± .95   | 1.19  | 1.78          |
|                                       | ♀ ♀ | 64  | <i>d</i>             | 110.3 ± .71    |             |   |               |
|                                       | ♂ ♂ | 82  | <i>D</i>             | 108.5 ± .61    | 2.7 ± .92   | 2.49  |               |
|                                       | ♂ ♂ | 79  | <i>d</i>             | 111.2 ± .69    |             |   |               |
| 4) ♀ F <sub>1</sub> wild by ♂ Little  | ♀ ♀ | 57  | <i>D</i>             | 149.1 ± .50    | 7.2 ± .67   | 4.83  | 3.31          |
|                                       | ♀ ♀ | 58  | <i>d</i>             | 156.3 ± .45    |             |   |               |
|                                       | ♂ ♂ | 46  | <i>D</i>             | 148.4 ± .66    | 2.2 ± .85   | 1.48  |               |
|                                       | ♂ ♂ | 41  | <i>d</i>             | 150.6 ± .53    |             |   |               |
| ♀ Little by ♂ F <sub>1</sub> wild     | ♀ ♀ | 80  | <i>D</i>             | 148.4 ± .50    | 2.5 ± .69   | 1.75  | 1.59          |
|                                       | ♀ ♀ | 84  | <i>d</i>             | 150.9 ± .48    |             |   |               |
|                                       | ♂ ♂ | 75  | <i>D</i>             | 146.1 ± .47    | 2.1 ± .70   | 1.44  |               |
|                                       | ♂ ♂ | 61  | <i>d</i>             | 148.2 ± .50    |             |   |               |

*bactrianus* animals were obtained by crossing ♀ Little by ♂ *bactrianus*. These along with the *bactrianus* strain were described by CASTLE et al (1936 II).

Comparison of dilute (*d*) and intense (*D*) backcross animals in the four

crosses described shows an apparent localized influence of  $d$  in the tail region. In all backcrosses and their reciprocals the dilute mice have a significantly higher number of tail rings. This holds true even in the ♀  $F_1$  by ♂ Gates cross where  $d$  is closely linked with  $se$ . In Cross 1 it was apparent that  $se$  exerted a contrary negative influence on growth as determined by a series of body measurements, evidently outweighing the dilution effect. In the tail region it seems that dilution outweighs the short-ear effect.

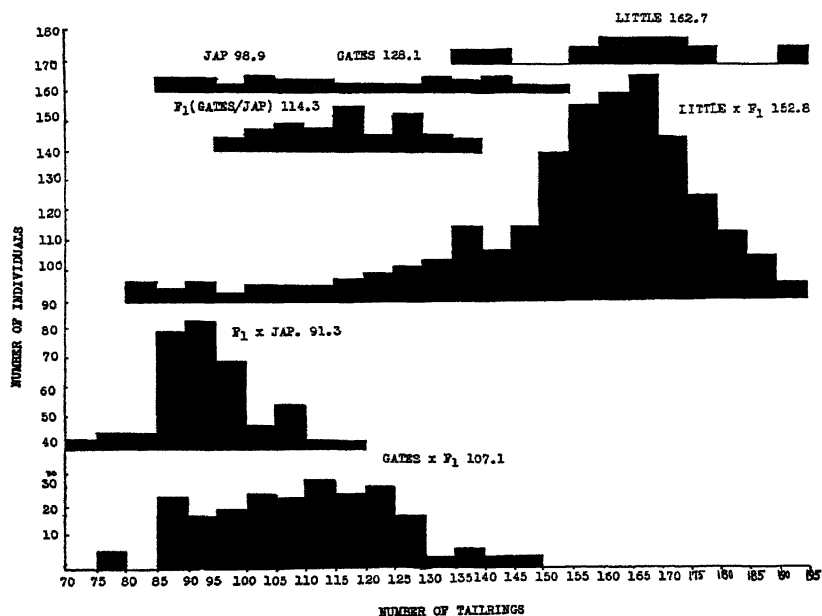


FIGURE 3.—Frequency polygons for the size character tail ring number in the parental,  $F_1$ , and backcross generations.

These results are expressed as percentage influences of dilution as measured by an increase over the average (see table 11).

In all crosses studied brown mice are larger than their dominant black sibs as regards body measurements, as well as in number of caudal vertebrae and length of 15th vertebra. There is no apparent association between the brown factor and number of tail rings in the crosses studied.

There is some evidence that the chromosome bearing non-agouti ( $a$ ) carries factors influencing the growth of tail rings. In the ♀  $F_1$  *bactrianus* by ♂ Little cross and its reciprocal, where the chromosome carrying  $a$  was introduced by the Little parent having the greater tail ring number, both males and females have a significantly higher tail ring number. In the cross of ♀  $F_1$  wild by ♂ Little and its reciprocal, where  $a$  was intro-

duced by the smaller parent, non-agouti males and females have a significantly lower mean tail ring number.

There can be found no support for the hypotheses of FORTUYN. Two lines of evidence indicate that tail ring number does not behave in a manner suggesting segregation of a single pair of autosomal factors. First, there is indication that factors favorable to the growth of tail rings are to be found in two or more chromosomes. Second, the backcross frequency

TABLE 12  
*The size character, tail ring number, in reciprocal crosses.*

| CROSS                                     | NO. | MEAN<br>♂ ♂   | NO. | MEAN<br>♀ ♀   | DIFF. MEANS<br>(SEX) |
|---|-----|---------------|-----|---------------|----------------------|
| ♀ F <sub>1</sub> by ♂ Little              | 62  | 149.28 ± 1.60 | 87  | 148.31 ± 1.40 | .97 ± 2.12           |
| ♀ Little by ♂ F <sub>1</sub>              | 557 | 155.46 ± 1.20 | 670 | 154.55 ± 1.10 | .91 ± 1.62           |
| Diff. Means (cross)                       |     | 6.18 ± 2.00   |     | 6.24 ± 1.78   |                      |
| ♀ F <sub>1</sub> <i>bact.</i> by ♂ Little | 279 | 94.81 ± .28   | 293 | 97.21 ± .36   | 2.40 ± .47           |
| ♀ Little by ♂ F <sub>1</sub> <i>bact.</i> | 163 | 109.8 ± .67   | 127 | 109.7 ± .53   | 0.1 ± .85            |
| Diff. Means (cross)                       |     | 15.0 ± .73    |     | 12.5 ± .65    |                      |
| ♀ F <sub>1</sub> by ♂ Gates               | 815 | 93.69 ± .83   | 59  | 94.74 ± .82   | 1.05 ± 1.22          |
| ♀ Gates by ♂ F <sub>1</sub>               | 819 | 108.40 ± .59  | 56  | 106.4 ± .66   | 2.0 ± .88            |
| Diff. Means (cross)                       |     | 14.71 ± 1.06  |     | 11.66 ± 1.09  |                      |
| ♀ F <sub>1</sub> (wild) by ♂ Little       | 136 | 149.2 ± .75   | 164 | 152.4 ± .70   | 3.2 ± 1.01           |
| ♀ Little by ♂ F <sub>1</sub> (wild)       | 87  | 147.0 ± .58   | 115 | 148.6 ± .55   | 1.6 ± .79            |
| Diff. Means (cross)                       |     | 2.2 ± .94     |     | 3.8 ± .89     |                      |

curves give no suggestion of bimodality (fig. 3). Likewise, the variability of the backcross generation over the parental strains is great. Also there is no evidence to support FORTUYN's suggestion of modifying genes in the Y chromosome.

From table 12 it can be seen that there are significant differences between male backcross populations in reciprocal crosses, but like differences are to be found between the female populations. Apparently this is a differential maternal effect. The mother having the highest tail ring number always produces offspring having higher tail ring number. If any differential effect of the Y chromosome is patent, male backcross offspring produced by the F<sub>1</sub> mothers in crosses 1, 2 and 4 should reveal this effect. Evidently this is not the case.

## ACKNOWLEDGMENTS

I wish to thank Dr. W. E. CASTLE who suggested this problem and provided most of the material, and Dr. E. M. EAST under whose direction the last part of the work was carried out.

## SUMMARY

A series of crosses was made using inbred strains of mice of known genotypes in the hope of obtaining further evidence concerning the nature of size genes.

Cross 1, a backcross between the Gates strain of mice and an  $F_1$  produced by crossings of ♀ Gates by ♂ Japanese mice, the reciprocal cross of one previously reported (CASTLE et al 1936 II), indicates that the association between pink-eye and smaller body size may not be a genuine phenomenon.

Brown backcross mice in two separate backcrosses are regularly larger than their black sibs, illustrating the same association reported by previous authors. In Cross 4 the chromosome carrying the gene for brown coat color came into the cross from a slightly smaller parent strain whereas in previous crosses the brown parent, although from the same strain of mice, was larger. The evidence from coupling and repulsion experiments along with the fact that all strains of brown mice so far tested have shown association between the brown gene and larger body size, suggests that the brown gene itself is influencing general body growth, simulating in action the dilution gene.

Backcross mice segregating for the short-ear gene are distinctly smaller than their normal ear sibs. A quantitative estimate of the effects of the short-ear gene could not be made, due to the small number of backcross mice obtained, but it is indicated that this gene is more influential in reducing size than the brown gene is in stimulating growth.

There is some indication that size genes making for smaller body size are to be found in the chromosomes marked by the piebald and waltzing genes.

The dilution gene has an apparent localized influence in the tail region. Data from separate backcrosses, with their reciprocals, show dilute mice to have a consistent and significant higher tail ring number than the intense sibs. An influence of the dilution gene was also noted as regards the growth of caudal vertebrae.

There could be found no evidence in support of FORTUYN's hypothesis that tail ring number behaved as though determined by a single pair of autosomal genes, nor of his hypothesis suggesting the presence of genes in the Y chromosome modifying the expression of this character.

A definite maternal effect was patent as regards the inheritance of tail ring number.

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# EFFECTS OF A COMPOUND DUPLICATION OF THE X CHROMOSOME OF DROSOPHILA MELANOGASTER

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Received March 14, 1938

## INTRODUCTION

DOBZHANSKY (1934) has described effects on crossing over and disjunction of duplicating fragments of the X chromosome of *Drosophila melanogaster*. He has found that fragments partially suppress crossing over between the entire X's and that the effect is correlated with the length of the fragment. Some of the fragments were free and controlled by their own spindle fiber attachments and some others were attached to an entire X chromosome at the spindle fiber end.

Duplication 100 described here differs in length and in composition from those described in DOBZHANSKY's study; the results are thus largely an extension of his results. The study was undertaken especially for a comparison of the effects of the same fragment in both states, when free and when attached to an entire X. Some effects of a Y chromosome in different combinations of two X's and the fragment have been observed.

## MATERIAL

The duplication arose spontaneously in an apricot male which was mated to an attached-X yellow female. It was found, December, 1922, as a single not-yellow daughter, sister to the numerous yellow daughters. As in similar duplications, which have frequently been obtained later by irradiation of males, the X derived from the male was a deficient chromosome; it contained a distal and a proximal section and lacked the middle section of a normal X. In this case the deficient sperm had fertilized an XX egg in which the attached X's both carried the gene for yellow; the extra fragment, that is, the duplication, carried only wild type alleles, consequently the duplication female was not-yellow. The formula is XXX<sup>pd</sup> (fig. 1 a). When the not-yellow female was crossed to an apricot male the duplication-bearing daughters received a Y chromosome from their father and were therefore XXX<sup>pd</sup>Y (fig. 1 b).

One not-yellow daughter of the original duplication female differed from the others in that the fragment had become attached to one of the whole X chromosomes received from the mother; she had received her second X from her father. The formula is XXX<sup>pd</sup> (fig. 1 c). A chromosome

having a fragment attached to it would have resulted if crossing over had taken place between the proximal section of the fragment ( $X^p$ ) and one of the attached X's. Such crossovers continued to occur rarely in the attached-X duplication stock ( $XXX^{pd}Y$ ), and the attached-duplication lines derived from them are known as Dp (1;1) 100. A small percentage of  $XXX^{pd}Y$  females (fig. 1 d) regularly occurs in these lines.

A fifth combination of the X homologues arose when an egg carrying the free duplicating fragment was fertilized by a rare type of non-disjunctional sperm carrying two paternal free X's. The formula is  $XXX^{pd}$  (fig. 1 e); the line is Dp (1;f) 100. The two X chromosomes in figure 1 e are marked with the symbols denoting mutant loci used in studying crossing over, to be described in detail below.

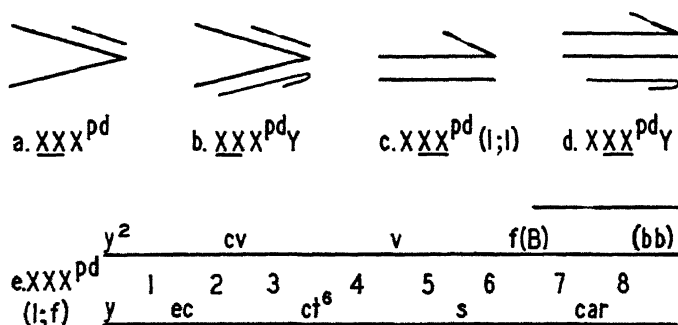


FIGURE 1.—Diagram representing the sex chromosomes (short line for  $X^{pd}$  or fragment, long line for X and hook for Y) in females of lines of Dp-100. Symbols for mutant characters used in crossover experiments and the numbers of the regions delimited by the mutant genes are entered in e.

From the Dp (1;f) line, in which the X's are not attached and the fragment also is free, it has been easy to determine genetically the extent of the deficiency of the fragment by observing the character of females carrying X's homozygous for mutant genes, and the wild type fragment. The distal section of the fragment ( $X^d$ ) carries normal alleles of seven tested mutant characters (*y*, *ac*, *sc*, *svr*, *sta*, *br*, *pn*) from yellow to prune inclusive. The proximal section ( $X^p$ ) carries normal alleles of fused (*fu*), carnation (*car*) and bobbed (*bb*). The fragment was found to be deficient for 16 tested loci in the middle of the chromosome including white to outstretched, vibrissae, small-eye and probably Beadex (*Bx*). There are exaggeration effects produced in achaete, facet and Beadex. Bar (*B*) eyes are narrower in duplication females whose X's are homozygous or heterozygous for Bar than they are in the respective non-duplication sisters, and also the eyes were very narrow in the few surviving duplication Bar males that have been observed.

The fragment is nearly always lethal in males. The rare survivors have been omitted from the tables. Females carrying the fragment show somatic effects of X chromosome duplication described by other authors such as narrow wings having straight outer margins and sometimes nicked or very serrated inner margins and tips. In extreme cases less than half of the wing remains, deeply indented. DOBZHANSKY and SCHULTZ (1931) found that intersexes carrying Dp-100 are of extreme female type, even breeding as female in one case of five that were tested.

Cytological preparations of oögonial cells have shown the duplication both in the attached, and in the free condition when the X's were free or attached. DOBZHANSKY (1932) measured the fragment in metaphase plates of oögonial divisions and found it to be about two-thirds of the length of an X, never as much as three-fourths. The measurements showed that the distal section of the fragment in metaphase plates comprises somewhat less than one-third of the length of the fragment. Measured by locations of genes on the salivary chromosome map, the proximal section of the fragment is about once and a half times as long as the distal section and the whole fragment is less than one-fourth of the length of the whole chromosome (fig. 2).

In an experiment (not used in this study) with a line of free X's and the fragment free ( $XXX^{pd}$ ), two female mosaics and two gynandromorphs were observed, and in a line of attached X's and free fragment one female mosaic was observed. They can all be explained by somatic elimination; in one gynandromorph both an X and the fragment had been eliminated together or at two different divisions in the formation of the male region.

#### CROSSING OVER BETWEEN ENTIRE X CHROMOSOMES

Frequency of crossing over has been studied in the two forms of the duplication in which the two entire X's are not attached to each other, that is, in the  $XXX^{pd}$  line, Dp (1;f) 100, and in the  $XXX^{pd}$  line, Dp(1;1) 100, and also in the latter line when a Y was present ( $XXX^{pd}Y$ ), (tables 6-10, Appendix).

Females of the constitution Dp/ $y^2cv$  *v f/y ec ct<sup>s</sup> car* were mated to *y ec cv ct<sup>s</sup> v s<sup>2</sup> f car bb<sup>1</sup>* ( $yX_9$ ) males, in alternated testcrosses, or were outcrossed to males marked by *B*; in experiment 7, one X was marked with *B* instead of *f*, and with *bb* to delimit the eighth region. Sisters of the duplication females were used as controls. In the (1;1) line the duplicating fragment was attached to the  $y^2cv$  *v f* chromosome. In the crossover experiments and in all others to be reported the entire X's of the duplication females have carried yellow (or  $y^2$ ); consequently when they were mated to males that were *y* or  $y^2$  the character not-yellow was an indicator of the presence of the fragment, except when exchange between X and the distal



section of the fragment ( $X^d$ ) had taken place. Such  $X^d/X$  crossing over has been observed only in a few males and in two females (in experiment 7) which were not yellow but were carnation or bobbed showing that the fragment ( $X^p$ ) was not present.

In testcrosses and in outcrosses of the line of the free fragment ( $XXX^{pd}$ ) females that were  $y^2$  but were wild type at other marked loci had been

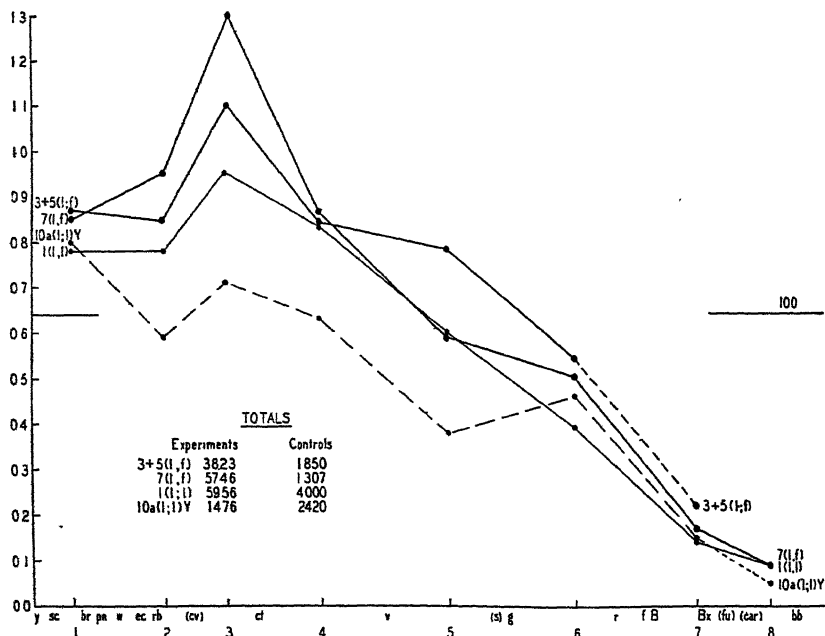


FIGURE 2.—Relation of percents of crossing over, in 8 regions between the two entire X chromosomes in Dp-100, to percents from controls (vertical axis) plotted against the salivary chromosome map (longitudinal axis). Relative positions of mutant genes on the chromosome (according to MACKENSON and BRIDGES) are marked by the symbols for the mutant characters (those in parenthesis have not been precisely located). The extent of the duplicating fragment is indicated by horizontal lines extending over the loci included in the distal and proximal components of the fragment. The fragment was free in experiments 3+5 and 7 and was attached to the proximal end of an X in experiments 1 and 10a; a Y chromosome was also present in experiment 10a. In experiment 3+5, half of the crossovers between the proximal section of the fragment and X were included in region 7.

derived from XX eggs fertilized by Y sperm. In outcrosses of the line of attached fragment occurred wild type duplication females which were derived from  $XXX^{pd}$  eggs fertilized by Y sperm. These two types may for convenience be called "exceptional females." They occur regularly (as do "exceptions" from XXY females from which the term is borrowed) but they are exceptions in the sense that they form much smaller classes than the females from X,  $XX^{pd}$  or  $XX^{pd}$  eggs.

The term "percent of crossing over" is used to denote the percent of times that crossing over has occurred among total chromatids represented by observed zygotes, and not the percent obtained directly from observed regular zygotes. This method has been chosen because all of the chromatids are involved in the mechanism of crossing over. Hence the percents of crossing over have been reckoned as of a total that includes the chromatids of "exceptional" gametes (whether they produce viable or lethal zygotes) and of gametes of other smaller classes. Exceptional zygotes that survive in the line of the free fragment ( $XXX^{pd}$ ), that is  $y^2$   $XXY$  females, represent four exceptional gametes (two containing the maternal X's and two containing the fragment, one of each fertilized by X or by Y sperm) since three of the four resulting zygotes, namely  $XXX$ ,  $XX^{pd}$  and  $X^{pd}Y$ , are lethal. When the total counts include half of the zygotes (for example, non-duplication males and females) the exceptional females count as two chromatids, or as one chromatid when only males or females that do not carry the duplication are counted. The chromatids of the exceptions were in some cases crossovers and were rated accordingly.

In lines of the attached fragment ( $XXX^{pd}$  and  $XX.X^{pd}Y$ )  $y^2$  females are crossovers between the proximal fragment and the free X; their X's are attached to each other and they represent two chromatids each, when the total includes non-duplication females and males (the lethal zygotes are pointed out in connection with  $X^p/X$  exchange). The exceptional females in these lines, from very infrequent  $XXX^{pd}-O$  segregation, are wild type duplication females ( $XXX^{pd}Y$ ) and are treated as are exceptions in the line (i; f).

Table 1 shows the percents of crossing over from exchange between entire X's and the relation of those percents to the corresponding percents from controls. These are also shown graphically in figure 2.

The most noticeable result is the marked reduction in X/X crossing over in the presence of the fragment, especially in the region homologous to the proximal section of the fragment. When a Y was present as well as the fragment attached to an X, crossing over between the X's was still further reduced (experiment 10a). The results will be discussed later.

#### CROSSING OVER BETWEEN THE PROXIMAL AND DISTAL SECTIONS OF THE FRAGMENT AND AN ENTIRE X

Conclusions in regard to the frequency of  $X^p/X$  crossing over have been pieced together from the results obtained in different kinds of experiments and are summarized in table 3.

In the testcross experiment 7, in which the fragment was free and one maternal chromosome was marked with  $bb$ , there were three unique types that have been used to allocate the ambiguous

TABLE 1

Percents of crossing over between entire  $X$ 's in  $Dp$ -100 in the presence of: experiments 3-7, the free fragment in  $XXX^{pd}$ ; experiment 1, the attached fragment in  $XXX^{pd}$ ; in experiment 10a, the attached fragment and  $Y$  in  $XXX^{pd}Y$ .

| REGION  | 1<br><i>y-ec</i> | 2<br><i>ec-cv</i> | 3<br><i>cv-cl</i> | 4<br><i>cl-v</i> | 5<br><i>v-s</i> | 6<br><i>s-f(B)</i> | 7<br><i>f(B)-car</i> | 8<br><i>car-bb</i> | TOTAL<br>CHROMATIDS |
|---------|------------------|-------------------|-------------------|------------------|-----------------|--------------------|----------------------|--------------------|---------------------|
| exp. 3  | 7.3              | 8.9               | 8.6               | 12.3             | 7.3             | 7.4                | 1.36*                | —                  | 2225                |
| control | 7.9              | 10.4              | 8.8               | 15.2             | 8.7             | 13.2               | 5.8                  | —                  | 1392                |
| ratio   | .93              | .86               | .98               | .81              | .84             | .56                | .22                  |                    |                     |
| exp. 5  | 6.3              | 8.1               | 9.2               | 14.2             | 6.3             | 5.8                | 1.3*                 | —                  | 1599                |
| control | 7.9              | 9.2               | 5.7               | 16.8             | 9.2             | 10.5               | 6.4                  | —                  | 458                 |
| ratio   | .80              | .88               | 1.6               | .85              | .69             | .55                | .20                  |                    |                     |
| exp. 7  | 6.2              | 8.6               | 10.7              | 13.9             | 5.5             | 7.4                | 1.13                 | 0.55               | 5682                |
| control | 7.3              | 9.0               | 8.2               | 16.2             | 9.4             | 14.8               | 6.7                  | 5.8                | 1307                |
| ratio   | .85              | .95               | 1.3               | .86              | .59             | .50                | .17                  | .09                |                     |
| exp. 1  | 4.7              | 7.6               | 8.1               | 13.0             | 6.5             | 5.4                | 1.0                  | 0.54               | 5956                |
| control | 6.0              | 9.8               | 8.6               | 15.6             | 10.9            | 13.8               | 7.1                  |                    | 4000                |
| ratio   | .78              | .78               | .94               | .83              | .60             | .39                | .14                  | .09†               |                     |
| exp 10a | 4.8              | 5.8               | 6.1               | 9.8              | 4.2             | 6.4                | 1.1                  | 0.27               | 1476                |
| ratio   | .80              | .59               | .71               | .63              | .38             | .46                | .15                  | .05†               |                     |

\* Includes one half the quota of  $X^p/X$  crossovers at 7; the other half is included among non-crossovers for region 7.

† Based on control of experiment 7.

TABLE 2

Experiment 7,  $Dp$  (1;f)/ $y^2 cv v B bb/y^2 ec cl^6 s car$  ♀ by  $yXg$  ♂. Allocation of observed types of crossovers.

| REGION              | X/X CROSSTOVERS |           |            | X <sup>p</sup> CROSSTOVERS |          | X <sup>d</sup> /X CROSSTOVERS |             |             |             |
|---------------------|-----------------|-----------|------------|----------------------------|----------|-------------------------------|-------------|-------------|-------------|
|                     | —, 7            | —, 8      | —, 7, 8    | 7                          | 8        | Dp<br>I                       |             | non-Dp<br>I |             |
|                     | .               | .         | .          | .                          | .        | $y^2$                         | $y$         | .           | <i>ec</i>   |
|                     | .               | .         | .          | .                          | .        | <i>cv</i>                     | <i>ec</i>   | <i>cv</i>   | <i>cl^6</i> |
|                     | .               | .         | .          | .                          | .        | $v$                           | <i>cl^6</i> | $v$         | $s$         |
|                     | $s$             | $s$       | $s$        | $s$                        | $s$      | $s$                           |             |             |             |
|                     | <i>B</i>        | <i>B</i>  | <i>B</i>   | <i>B</i>                   | <i>B</i> | <i>B</i>                      |             | <i>B</i>    |             |
|                     | <i>car</i>      |           | <i>car</i> |                            |          |                               |             |             | <i>car</i>  |
|                     | <i>bb</i>       | <i>bb</i> | <i>bb</i>  |                            |          |                               |             |             |             |
| ♀ ♀ observed unique | 37              | 27        | 11         | 0                          |          | 1                             |             | 1           | 1           |
| ambiguous           |                 | 27        |            |                            | 5        |                               |             |             |             |
| ♀ ♀ observed and    |                 |           |            |                            |          |                               |             |             |             |
| allocated†          | 37              | 27        | 20         | 11                         | 0        | 5                             | 5           | 2           | 2           |
| ♂ ♂ observed unique | 46              |           |            |                            |          | 1                             | 0           | 1           | 1           |
|                     |                 |           |            |                            |          |                               |             | 2           | 1           |

† Allocations are printed in bold-faced type.

types of crossovers due to exchange in the region homologous to the proximal component of the fragment. Crossovers were determined in non-duplication female offspring.

Of the two complementary types of double crossovers between entire X's at 7 and 8, one, which is *B car bb*, is unique and none of this type was observed (table 2), as expected for so short a section (mutant characters to the left of *s* are disregarded since they varied with distal crossing over between the X's which is irrelevant to the present discussion). The complementary type (*s not-B not-car not-bb*) is ambiguous since it is also the type that would result (more often) from

TABLE 3

*Corrected percent of crossing over involving the fragments and entire X's in Dp-100, and in other duplications from the data of DOBZHANSKY (1934) and PHILIP (1934). Percents for experiment 7 are based on allocations shown in table 2.*

| KIND OF CROSSOVER |                                 | X/X    |      | X <sup>p</sup> /X |                   |                   |   | X <sup>d</sup> /X |
|-------------------|---------------------------------|--------|------|-------------------|-------------------|-------------------|---|-------------------|
|                   |                                 |        |      | X-EGGS            |                   | XX-EGGS           |   |                   |
|                   |                                 | REGION | 7    | 8                 | 7                 | 8                 | 7 | 8                 |
| No. of Exp.       | Constitution of ♀               |        |      |                   |                   |                   |   |                   |
| 7                 | XXXX <sup>pd</sup>              | 1.13   | 0.55 | 0.18              | 0.07              | —                 | — | 0.04              |
| 9d                | XXXX <sup>pd</sup>              |        |      | 0.10              | —                 | 0.19              | — |                   |
| 9                 | XXXX <sup>pd</sup>              |        |      | 0.04              |                   | 0.10              |   | 0.01              |
|                   |                                 |        |      |                   | X <sup>p</sup> /X |                   |   |                   |
|                   |                                 |        |      |                   | with att. X       | with free X       |   |                   |
| 1                 | XXX <sup>pd</sup>               | 1.0    | 0.54 |                   | —                 | 0.02              |   | none              |
| 1d                | XXX <sup>pd</sup>               |        |      |                   | none              | 0.06              |   | none              |
| 10a               | XXX <sup>pd</sup> Y             | 1.09   | 0.27 |                   | 0.15              | 0.15              |   | none              |
| 10b               | XXX <sup>pd</sup> Y             |        |      |                   | 0.08              | 0.06              |   | none              |
|                   |                                 |        |      |                   |                   | X <sup>p</sup> /X |   |                   |
| Dp-138            | XXX <sup>p</sup>                | 1.24   | 0.15 |                   |                   |                   |   |                   |
|                   | XXX <sup>p</sup> Y              |        |      |                   |                   | 0.7               |   |                   |
| Dp-B <sup>s</sup> | XXX <sup>p</sup> i <sup>4</sup> |        |      |                   |                   | 0.14              |   |                   |
|                   | XXX <sup>p</sup>                |        |      |                   |                   | 0.20              |   |                   |
| Dp-105            | XXX <sup>pd</sup> Y             |        |      |                   |                   |                   |   | ?                 |
|                   | XXX <sup>pd</sup>               |        |      |                   |                   |                   |   | 0.11              |
| Dp-102            | XXX <sup>pd</sup>               |        |      |                   |                   |                   |   | 0.23              |
| Dp-T-3            | XXX <sup>d</sup> i <sup>3</sup> |        |      |                   |                   |                   |   | 0.21              |

\* Frequency much lower than frequency of X<sup>p</sup>/X in Dp-138.

exchange at 7 between the fragment and the X carrying *s* and *car*. In the absence of the unique 7, 8 double crossover type, the 5 females of the sable type are all allocated to the class of X<sup>p</sup>/X crossover at 7. The same *s* (*y* or *y*<sup>2</sup>) type might also be a duplication female in which the fragment was yellow, from exchange between an X and the distal section of the fragment. X<sup>d</sup>/X crossovers are rare, and none of the sable (*y* or *y*<sup>2</sup>) females is allocated to this class. A *y*<sup>2</sup> *cv v B* female of the complementary type showed by somatic characteristics of duplication that she was of the class.

Another unique type is one of the two complementary X/X crossovers at 8, namely, *s car bb* females. Eleven of these were observed and 27 of the complementary type which is *B not-car not-bb*. The latter is ambiguous, resulting also from exchange between the fragment and the X carrying *B*, at 7 or at 8. Five of the ambiguous *B* females are allocated to the class of X<sup>p</sup>/X crossovers at 7 corresponding to the 5 of the complementary sable class described above. Finally, 2 of the 27 have been rated as X<sup>p</sup>/X crossovers at 8 on the basis of the ratio of X/X crossovers at 8 to those at 7. This leaves 20 females that were Bar in the class of X/X crossovers at 8. Of the total

of 27 *B not-car not-bb* females, 10 were tested and found not to be of the class carrying a duplication that was yellow, from  $X^d/X$  crossing over, and six others were examined and showed no somatic characters of duplication. The ratio of the 11 observed complementary unique type, *s car bb*, to the 20 *B not-car not-bb* flies that were rated as  $X/X$  crossovers at 8 is in fair agreement with the observed 0.65:1 ratio of *bb* to *not-bb* among the non-duplication flies of the experiment. The rating of 2 of the *B not-car not-bb* females as  $X^p/X$  crossovers at 8 has led to allocation to the complementary  $X^p/X$  class (which shows *s* and *car*) of 2 females from the ambiguous non-crossover class (*y ec ci<sup>6</sup> s car*).

Percents of crossing over in X gametes between the free fragment and the X chromosomes have been obtained from the allocations of the observed types in experiment 7 (tables 2 and 3).

When the fragment is free, exchange between  $X^p$  and an X transfers the whole chromatid from its own spindle-fiber attachment to that of the fragment and vice versa (fig. 4). If the chromosomes separate after crossing over, an X chromatid and a fragment pass to each pole; if the other X segregates at random X and XX eggs containing the crossover chromosome are to be expected with equal frequency after exchange between X and the proximal component of the fragment.

In experiment 7 exchange was detectable only in X gametes. To test the presence of the crossover X in one-X and two-X eggs when  $X^p/X$  exchange had occurred, duplication females whose X's were marked by *car bb* or by *bb<sup>1</sup>/car bb<sup>1</sup>* were mated to  $y^2 bb^1 Y^{bb}$  males when the only source of *not-bb* was the fragment. Experiments 9 and 9d show that both kinds of eggs occurred (table 10 at (c), appendix). The  $Y^{bb}$  in experiment 9d had too slight an effect on *bb<sup>1</sup>* for exchange to be detectable in region 8, but the distribution of *car* showed that crossover chromosomes from exchange between the fragment and X in region 7 were present in both kinds of gametes.

A different  $Y^{bb}$  (and *bb<sup>1</sup>* in both maternal X's) was used in experiment 9. The values were lower than the corresponding values in experiments 7 and 9d, owing probably to the action of *bb<sup>1</sup>*; heterozygous *bb<sup>1</sup>* is known to reduce crossing over in XX females increasingly toward the proximal end, the amount of crossing over between *car* and *bb* being about half of normal.

In calculating the percents of  $X^p/X$  crossing over in X and XX gametes (table 3) corrections were made for lethal zygotes (XXX,  $XX^{p^d}$  and  $X^{p^d}Y$ ). The differences between the frequencies of X and XX gametes containing  $X^p/X$  crossovers are not significant, though the XX gametes were consistently more frequent than the X gametes. Equal frequencies are (as shown above) to be expected if the fragment and X go to opposite poles after exchange between them, and distribution of the non-crossover X is random.

When the fragment is attached to an X, reciprocal exchange between them cannot be detected. In testcrosses (experiment 1) non-reciprocal

(diagonal) exchange would produce attached-X females (XXY). They would be of the type of XX females that were crossovers between the X's, that is  $y^2 cv v f$  (if single crossovers) since the fragment was attached to the X so marked. The males that were  $f$  (and showed other recessive mutant characters) would have been derived from X/X exchange only, and are expected to be as frequent as the females of the same origin. The ratio of the apparent crossovers, at 8, is 14 females to 18 males, showing no excess of females attributable to exchange with the fragment.

In the outcrosses to males having Bar eyes (experiments 1d, 10a, 10b) diagonal crossovers would be attached-X females of a type that is not ambiguous (since the other class would be heterozygous for  $B$ ); they would be  $y^2 cv v f$  (or  $y w cv v f$  in experiment 10b from the second kind of mating). If exchange had taken place also between the X's, the attached X females might be  $y^2$  ( $y$  or  $yw$  in part of experiment 10b) and wild type in other distal regions. The percent of crossing over involving  $X^p$  and the attached X was obtained from the observed frequency of all such females (showing the proximal recessive characters). Each one represents eight chromatids, when correction is made for lethal classes of zygotes (from attached-X gametes fertilized by X sperm and from gametes carrying two fragments attached to each other) and correction for reciprocal  $X^p/X$  crossovers of an ambiguous type and the lethals that they represent.

Crossing over between  $X^p$  and the free X in both testcrosses and outcrosses would produce  $y^2$  females that would be wild type for proximal loci but might from exchange between the X's show other distal recessive characters besides  $y^2$ . The percent of crossing over involving  $X^p$  and the free X was accordingly obtained from the observed frequency of proximally wild type attached-X females. Each one represents four chromatids when correction is made for lethals (zygotes from XX gametes fertilized by X sperm and zygotes from the complementary type of gamete containing a fragment).

To compare frequencies of exchange in the three combinations of the sex chromosome homologues, table 3, experiment 7 (in which  $bb'$  was not used) can be considered for the  $XXX^{pd}$  line. It is assumed, from the results of the other experiments with the free fragment, that XX gametes after  $X^p/X$  exchange in experiment 7 were at least as frequent as X gametes. Frequency of  $X^p/X$  crossing over was then 0.5 percent.

The results are not entirely satisfactory, owing to complications incidental to the use of  $bb'$  and of  $bb$  in X and Y, to the absence of some unique classes, and to the small sizes of the classes to be measured. They are however consistent, and appear to be sufficiently accurate to show an expected decrease in crossing over when  $bb'$  is involved, which is correlated with an observed increase in non-disjunction of X's. If the classifications

in the various experiments and the distribution shown in the table of allocations were correct, the results would indicate: that  $X^p/X$  crossing over occurs more frequently when the fragment is free than when it is attached to an X and a Y is not present; that Y increases  $X^p/X$  crossing over between an attached fragment and an X; and that when the fragment is attached, crossing over of the fragment is equally frequent with the attached X and with the free X (assuming that reciprocal exchange with the attached X and diagonal exchange are equally frequent).

Some of the  $X^p/X$  crossovers were at the same time crossovers in a more distal region between the entire X's. In experiment 7, with the line of the free fragment, 3 of the 5 observed  $X^p/X$  crossovers at 7 from X eggs were also crossovers in region 1 between the X's, (classified in table 8, appendix, as 7, 8 and 1, 7, 8 X/X crossovers, and allocated in table 2 to  $X^p/X$  crossovers). In the line of the attached fragment (with and without a Y) there were 10 (7 tested)  $X^p/X$  crossovers recovered in attached-X females. Of those 5 were also X/X crossovers in regions from 1 to 3 or 4 (experiments 1, 1d, 10a, 10b, tables 9 and 10). The second exchange (distal and between the X's) will be discussed in relation to disjunction.

Crossing over has not been studied between the Y and any of the X homologues.

There was a small class of yellow heterozygous bar extremely bobbed females in one of two experiments combined in experiment 9 (table 10, appendix). This type of female would be expected if an X gamete were fertilized by non-disjunctive XY sperm. Such sperm is frequent when  $bb'$  is present. It is not clear why these females were more extremely bobbed than the regular so-called "exceptional"  $y\ bb'Y^b$  females. The work of STERN, NEUHAUS and others on different effects of bobbed and on the properties and crossing over of the Y chromosome show that the account of Dp-100 is not complete without more accurate study of the effects of bobbed and the role of Y.

Crossovers between the distal component of the fragment and an X are very rare. Among the flies on which percentages were based, in the six experiments in which the chromosomes were suitably marked, only two such crossover females were observed among 5682 females in experiment 7 (table 2).

#### DISJUNCTION

##### XXX<sup>pd</sup>

Offspring of the original Dp-100 female are shown in table 4. Two tested apricot males were sterile, as expected in the absence of a Y chromosome in the mother. The yellow male is accounted for if an egg containing an X chromatid, resulting from exchange between the proximal section

of the fragment ( $X^p$ ) and an X, had been fertilized by Y sperm. The complementary crossover is a chromatid having the fragment attached to it in place of the other X. One of the five not-yellow females carried such a chromosome. The only other fertile not-yellow female, and probably three that died, arose from non-disjunctional eggs carrying the attached X's and the fragment. Such females were expected to be as frequent as the apricot XO males (32) derived from nullo-X eggs. The marked deviation from equality may be accounted for by the relative inviability of the

TABLE 4

*Offspring of the original  $XXX^{pd}$  (wild type duplication) female and of  $XXX^{pd}Y$  (wild type duplication) female descendants (the entire X's were homozygous for yellow).*

| PARENTAGE                         | NOT $X^p$ OR Y CROSSOVERS |   |       | $X^p$ OR Y CROSSOVERS |       |
|-----------------------------------|---------------------------|---|-------|-----------------------|-------|
|                                   | FEMALES                   |   | MALES | FEMALES               | MALES |
|                                   | y                         | NOT-YELLOW                                  | $w^a$ | y                     |       |
| NOT-YELLOW<br>$XXX^{pd} \text{♀}$ | $XXY$                     |   | XO    |                       | XY    |
| by $w^a \text{♂}$                 | 92                        | 1 $XXX^{pd}Y$<br>1 $XXX^{pd}(Y?)$<br>3 died | 32    |                       | 1     |

| FEMALES             |       |             |             |            |                  |                          |          |
|---------------------|-------|-------------|-------------|------------|------------------|--------------------------|----------|
| NOT-YELLOW          | y     | not-y       | w f B       | w f B      | f B ( $\pm yw$ ) | het. B                   | y het. B |
| $XXX^{pd} \text{♀}$ | $XXY$ | $XXX^{pd}Y$ | $XXX^{pd*}$ | $XX^{pd*}$ | XY               | $XXX^{pd}$ or $XXX^{pd}$ | XX       |
| by f B ♂            | 111   | 274         |             |            | 442              | 1¶                       |          |
| by y w f B ♂        | 428   | 859         | 1           | 2          | 1246             | 4†                       | 3‡       |

\* With or without a maternal Y chromosome

¶ Tested  $XXX^{pd}$ .

† Not tested.

‡ 2 tested.

duplication females which, in the earlier generations of the line, was associated with marked exaggeration of some of the somatic disturbances characteristic of duplication.

At disjunction the attached X's and the fragment went together to the same pole in 25.8 percent of the tetrads, the percent being represented by the ratio of non-duplication (XO) males to total non-duplication males and females (fig. 3b).

### $XXX^{pd}Y$

The  $XXX^{pd}Y$  line was derived from the non-disjunctional not-yellow daughter of the original  $XXX^{pd}$  female which had been mated to an XY  $w^a$  male. In this line there was a high percent of association at meiosis of attached-X's and fragment as shown by the high frequency of  $XXX^{pd}$  and



Y gametes which produced wild type duplication females and  $f B (\pm yw)$  males respectively when the fathers carried  $f$  and  $B$  (table 4).

The heterozygous  $B$  female whose father had been  $f B$  was shown by testing to have carried a maternal X with the fragment attached to it, the result of exchange between the fragment and an X. The two tested  $y$  heterozygous  $B$  females carried a single maternal X marked by  $y$ , presumably derived from exchange between the Y and an X, as observed by KAUFMANN (1933) in attached-X lines. From the matings to  $y w f B$  males at least two of the four not tested heterozygous  $B$  females showed somatic disturbances due to the presence of the fragment. The fragment may have been attached to an X (from  $X^p/X$  exchange) or it may have been present in the free state with a single maternal X which had resulted from exchange between the Y and an X.

The  $w f B$  female came from an egg containing the fragment ( $\pm Y$ ) fertilized by non-disjunctional sperm carrying two X chromosomes. With this fly originated the line of two free X's and a free fragment ( $XXX^p$ ).

In the  $XXX^pY$  line the sex chromosome complement is composed of three partially homologous units. In this respect it is similar to  $XXY$  females. The classical theory of secondary non-disjunction suggested by BRIDGES (1916) to account for the types of segregation observed in such females has until recently been generally accepted. The theory postulates that when two of the three partially homologous chromosomes, X, X and Y, pass to the opposite poles of the meiotic spindle the third goes at random with one or the other. If  $p$  represents the proportion of tetrads in which the two X's segregate away from the Y, the limiting value of  $p$  is 0.5. STURTEVANT and BEADLE (1936) have found that the value of  $p$  is 0.63 in  $XX^{1a}Y$  females which are heterozygous for the delta-49 inversion in X, and STURTEVANT (1936) has found in triplo-IV flies that the frequency of one type of segregation of the fourth chromosomes may be as high as 70 percent. Likewise in some lines of attached X's with X-duplication, DOBZHANSKY (1934) and STURTEVANT (1936) have found a frequency of one type of segregation somewhat in excess of 50 percent.

The difference between this situation and the heretofore accepted theory of secondary non-disjunction in  $XXY$  is illustrated by STURTEVANT's diagram of triplo-IV segregation; according to the older view when two chromosomes, such as X and Y in an  $XXY$  female (A, B fig. 3a), go to opposite poles of the meiotic spindle, segregations II and III will occur with equal frequency and it follows that neither can exceed 50 percent of total segregation; the higher values of certain segregations obtained in the cases cited show that frequency of segregation may be independent of a limiting random distribution of the third element.

This is shown also in the  $XXX^pY$  line of Dp-100. The frequency of

segregation III (fig. 3c), by which the attached X's and the fragment go to the same pole, is represented by the ratio of non-duplication males, which are recovered from one fourth of the gametes from segregation III, to the total of non-duplication males and females, the latter being recovered from one fourth of the gametes from segregations I and II. The value is .76, corresponding to 76 percent of segregation III.

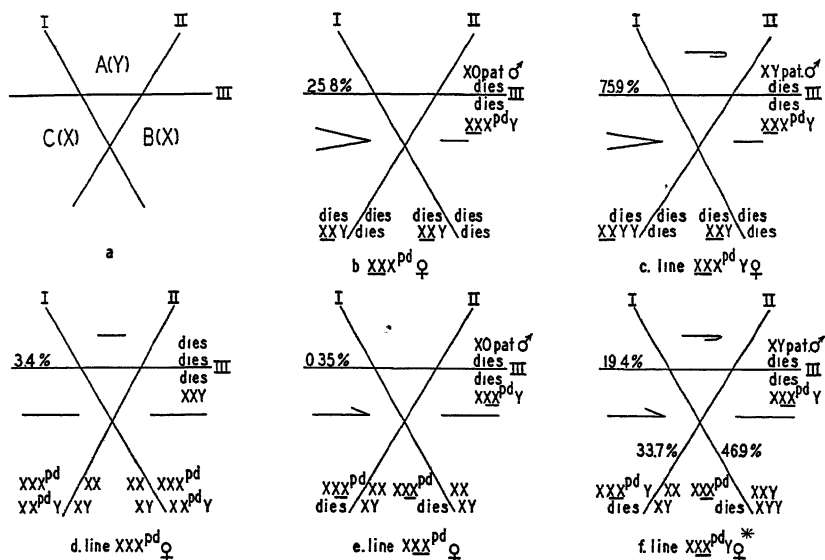


FIGURE 3.—Modeled after STURTEVANT's diagram of triplo-IV segregation to show types of segregation in different lines of Dp-100. Chromatids that pass to opposite poles are represented by symbols (long line for X, short line for fragment and hook for Y) on opposite sides of the lines of segregation (I, II and III). Zygotes resulting from eggs that receive the chromatids indicated by the symbols are noted on the same side of the line of segregation, first those from eggs fertilized by X sperm, second by Y sperm. Crossovers involving the fragment or Y are disregarded. Percents refer to frequencies of segregations.

\* Percents of segregations are recorded as found in experiment 10a and in agreement with those of experiment 10b. Other experiments showed a random segregation of Y with X and  $XXX^{pd}$  (I and II).

Unpublished data of Dr. SCHULTZ obtained from experiments with a duplication similar to Dp-100 show 60.7 percent of segregation III, 35.0 percent of segregation I and 4.3 percent of segregation II. The duplication (known as Dp (1:f) eq) carries normal alleles of  $y$  to  $pn$  (inclusive) and  $bb$ ; the proximal section is shorter than that of Dp-100.

$XXX^{pd}$ ,  $XXX^{pd}$  and  $XXX^{pd}Y$

In the other three lines, in which the entire X's are not attached to each other, the segregation by which the two X's go to the same pole of the

meiotic spindle is called segregation III. The values obtained are given in table 5.

In the line  $XXX^{pd}$  (fig. 3 d) only one of the four gametes from segregation III is recovered (as an  $XXY$  female which is yellow). Hence each observed zygote from segregation III represents two gametes among the flies of the classes that were counted, when they were one half of the total (for example non-duplication males and females). In that case the formula for deducing the frequency of total exceptional gametes in  $XXY$  females from the frequency of observed exceptional zygotes can be used. The proportion of exceptional gametes ( $= p$ ) from  $XXY$  females is equal to  $2q/1+q$ ,  $q$  representing the proportion of observed zygotes from  $XX$  and  $Y$  gametes. Applied to  $XXX^{pd}$  females this equation becomes  $p=q$  when non-duplication males or females only are counted, that is one fourth of the zygotes ( $XX$  or  $XY$ ) from  $X-XX^{pd}$  segregations (I and II), with one fourth of the zygotes ( $XXY$  females) from  $XX-X^{pd}$  segregation (III).

The same formula applies to the  $XXX^{pd}$  and  $XXX^{pd}Y$  lines. In these lines females from segregation III (fig. 3 e and f) are matroclinous duplication females and carry a  $Y$ . The males from segregation III can also be used if the paternal  $X$  is of good viability in  $XO$  or in  $XY$  individuals as was true in experiment 10a. Using both male and female exceptions with half of the other classes  $p$  equals  $q$ . Values thus obtained for segregation III (when two  $X$ 's segregate together) have been compared with those from controls and from  $XXY$  females (table 5).

In the  $XX$  control of experiment 7 no females from  $XX$  gametes were observed. Two controls from the same stocks were experiments 4 and 6a (experiment 6 and additional matings); the difference between the values obtained from these is not significant.

Values used for comparison of  $XXY$  females have been computed from the data of BRIDGES (1916, table 2), from the published data of BRIDGES and OLBRYCHT (1926) (adding thereto the number of exceptional males (87) and females (159) and a correction for relative inviability of patroclinous males supplied by Dr. BRIDGES), and from experiment 5n in which non-disjunction was measured in  $F_1$   $XXY$  females of experiment 5a.

In the lines in which the  $X$ 's were not attached to each other the percents of segregation of the entire  $X$ 's to the same pole are of three orders (table 5).

When the  $X$ 's are separate and the fragment is attached to one  $X$  ( $XXX^{pd}$ ) the frequency is slightly higher than the average of the more variable frequencies of primary non-disjunction in  $XX$  females of the control and other experiments. When a  $Y$  is also present ( $XXX^{pd}Y$ ) the  $X$ 's (one carrying the fragment) segregate together in about 19 percent of tetrads (experiments 10a and b).

In the line of the free fragment the frequencies are of the order of the more variable frequencies of XX-Y segregation in XX<sup>Y</sup> females; the similarity in frequency will be shown later to be superficial. When the X's carried *bb'* non-disjunction was increased (experiment 9).

In the form of Dp-100 which consists of a fragment attached to X, a free X and a Y (XXX<sup>pd</sup>Y) frequencies of three segregations were measured and the percents are shown in figure 3f. Percents of segregations I and II were estimated from a sample of 170 F<sub>1</sub> females (of experiment 10a) with and without the attached fragment. These females were tested for a Y chromosome by outcrossing and observing the occurrence of exceptional offspring. The results agreed closely with those obtained from a sample of

TABLE 5

*Percents of types of segregation in Dp-100 (and derived lines) and controls.*

| SEGREGATION                   | XX-O                        |                             | <u>XXX</u> <sup>pd</sup> -O |                             | XX-Y                                     | XX-X <sup>pd</sup>  |      |
|-------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|--|---------------------|------|
| Genetics of Drosophila (1925) | 0.10                        | exp. 1d                     | 0.40                        |                             | 8.2†                                     |                     |      |
| exp. 2                        | 0.30                        | exp. 1                      | 0.30                        |                             | 6.0¶                                     |                     |      |
| exp. 4                        | 0.58                        |                             |                             |                             |  | exp. 3*             | 3.50 |
| exp. 6a                       | 0.07                        |                             |                             | exp. 5n                     | 2.8                                      | exp. 5a*            | 4.30 |
| exp. 8                        | 0.0                         |                             |                             |                             |  | exp. 7*             | 3.35 |
|                               |                             |                             |                             |                             |  | exp. 9d             | 2.78 |
|                               |                             |                             |                             |                             |  | exp. 9              | 5.37 |
|                               | <u>XXX</u> <sup>pd</sup> -O | <u>XXX</u> <sup>pd</sup> -Y |                             | <u>XXX</u> <sup>pd</sup> -Y | X <sup>Y</sup> - <u>XX</u> <sup>pd</sup> | X-X <sup>pd</sup> Y |      |
| Dp-100                        | 25.8                        | 75.9                        | exp. 10a                    | 19.4                        | 46.9                                     | 33.7                |      |
|                               |                             |                             | exp. 10b                    | 18.5                        | 54.9                                     | 26.6                |      |

\* Includes XX gametes containing X<sup>p</sup>/X crossovers.

† Bridges (1916) table 2.

¶ Bridges and Olbrycht (1926).

171 F<sub>1</sub> females, in a similar experiment (10b), which were tested by outcrossing to Plum used as a detector of Y. Both tests seemed to show that Y segregates more often with X than with X and attached fragment, the ratios being about 1.4:1 and 1.7:1. However a subsequent test, in which XXX<sup>pd</sup>Y females of the stock used in experiment 10b were mated directly to Plum, showed the presence of an extra Y in only about half of the F<sub>1</sub> non-duplication females and males. The same random distribution of Y has again been found. Non-duplication male and female offspring of XXX<sup>pd</sup>Y females were mated to flies that were *y w* Dp *w*<sup>sc</sup> *co*<sup>b</sup>, another detector of Y. Of 217, tested in this way, one half of them (109) had received a Y chromosome from the mother, showing random distribution of Y with X, and with X and attached fragment in the XXX<sup>pd</sup>Y females whose offspring were tested. The question then remains open as to whether

Y segregates less frequently with an X when a fragment is attached to the X.

Equational non-disjunction had occurred in possibly four instances in the lines both of the free and of the attached fragment (see table 10 for parentage). In experiment 9 with the (1;f) line, a  $y^2 s car bb$  female was tested and gave  $y ec ct^s s car$  and  $y^2 cv v s car$  males (and crossovers), showing that the tested female had originated from non-disjunction of the proximal ends of the X's, one of the X's being a crossover in region 5. A  $y^2 cv v f$  female in experiment 5a had probably been derived from equational non-disjunction; or it may have come from reductional non-disjunction with X/X crossing over in region 7, that is, in a region nearer to the spindle-fiber attachment than in any other fly from reductional non-disjunction. In experiment 1d with the (1;1) line, a  $y w$  female was tested to determine whether she carried attached X's from  $X^p/X$  crossing over (with a second crossover between X's), but the test showed two separate X's and a Y chromosome. A  $y w$  female in experiment 10b was subject to the same explanation but was not tested.

Two patroclinous females were observed in experiment 10b. One was bred and the offspring showed that she had received two separate X's from her father and a Y from her mother. The females had developed from eggs of segregation III (figure 3f) but were of a class of zygotes so rarely to be expected that it has not been shown in the figure.

#### RELATIONS BETWEEN CROSSING OVER AND DISJUNCTION

The results of ANDERSON (1929), DOBZHANSKY (1933) and others have furnished abundant evidence that chromosomes that have exchanged segments usually pass to opposite poles of the meiotic spindle. Almost all of the recovered X/X crossovers in the experiments with  $Dp (1; f) 100$  have been found in zygotes from X eggs. Very rarely flies derived from XX eggs, known not to contain a crossover with the fragment, were equational for recessive mutant characters. For example, in experiment 9 only one of 594 XXY females that were reductional exceptions and not  $X^p/X$  crossovers was equational for distal loci. She was  $y^2 cv v bb$ , which indicated crossing over in region 5 in one X. There were no reciprocal crossovers in 23  $y^2$  females that were tested.

In the line of attached fragment without Y ( $XXX^{pd}$ , experiment 1, table 9) no equationals were observed among the very few females (9) derived from  $XXX^{pd}$  eggs.

In experiments 10a and 10b in which the mothers carried the attached fragment and Y, five females (namely two  $w$  and three  $w cv$ ) among 1195 exceptions from  $XXX^{pd}$  eggs were equational for distal loci, and one was a reciprocal crossover between X's (table 10 at (c), appendix); 183 of the

exceptional wild type duplication females were tested and were not reciprocal crossovers.

The effect on the distribution of the chromatids of a distal exchange between the X's accompanying  $X^P/X$  exchange was studied. When exchange occurs between the proximal section of a free fragment and an X it is assumed that the exchange will usually be followed by disjunction.

In the lines with the attached fragment ( $XXX^{Pd}$  and  $XXX^{Pd}Y$ ) exchange between the proximal section of the fragment and an X produces attached-X females (except in case of the undetectable exchange between the fragment and the X to which it attached). The distribution of the products of a second exchange between X's is determined by the attachment of the X's. Such double crossovers were found in about half of the attached-X females which were  $X^P/X$  crossovers.

By diagonal exchange between the fragment and X, sister strands are attached together and a resulting attached-X female shows the recessive characters of the X that is attached to the fragment and is detectable as an exceptional type in outcrosses. Sister strands being attached to each other a more distal exchange could take place only with the free X. There was one  $y w cv v f$  tested attached-X female (in experiment 10b) in which there had been no crossing over between X's. In two other such females a second exchange had occurred (in region 4 in one  $y^2 v f$  female in experiment 10a, and in region 1 or 2 in one  $y^2 cv v f$  female from the second type of female parent used in experiment 10b, table 10, appendix).

When  $X^P/X$  crossing over occurs between the fragment and the free X, the resulting attached-X female is  $y^2$  and a second crossover might be reciprocal (detectable only by testing and none were found) or equational and detectable by homozygosis of distal recessive genes (except homozygosis of  $y^2$ ). Of four  $y^2$  females, two in experiment 10d and one in experiment 10a (table 10, appendix) were tested and were probably not crossovers for X, and one  $y^2$  female in experiment 1 (table 9, appendix) was not tested. Three other crossovers between the fragment and the free X were also equationals for X; two  $y^2 cv$  females in experiment 10b were not tested but were of the type of attached-X females which are X/X crossovers in region 3 or 4, and one tested  $y^2$  female in experiment 10a carried attached X's of which one was a crossover in region 2 (she was homozygous for  $y^2$  and carried only normal alleles of *ec*).

It has been seen that when the X's and the fragment are free, crossover chromatids from exchange between the proximal section of the fragment and X are recovered in both X and XX eggs (table 3, experiment 9d, 9) and that three of the five females from X eggs found to be  $X^P/X$  crossovers, in experiment 7, were also X/X crossovers, indicating that about half of  $X^P/X$  crossovers were also crossovers between X's in the line of the free as in the line of the attached fragment.

The  $X^P/X$  crossing over is detectable only in females from X-gametes, in the experiments that show X/X crossing over, and is not detectable in XXY females from XX-gametes hence a calculation has been made of the frequency of distal X/X crossovers to be expected among XXY females when double crossing over ( $X^P/X$  and X/X) has occurred.

Assuming that, at the reductional (first) division, crossover proximal fragment and X pass to opposite poles, an X egg would receive a double

crossover ( $X^p/X$  and  $X/X$ ) chromatid only after three-strand double exchange and only when the crossover  $X$ 's also disjoined and passed to opposite poles. The double crossover chromatid would go to the haplo- $X$  pole, and after the equational division both daughter nuclei would receive a double crossover chromatid. The diplo- $X$  pole of the first division is represented by figure 4,  $A_1$ ; after the second division one daughter nucleus would receive a crossover and a non-crossover chromatid, and one would receive two non-crossover chromatids (the possible combinations indicated in figure 4,  $A_1$ ). The females recovered from eggs containing one

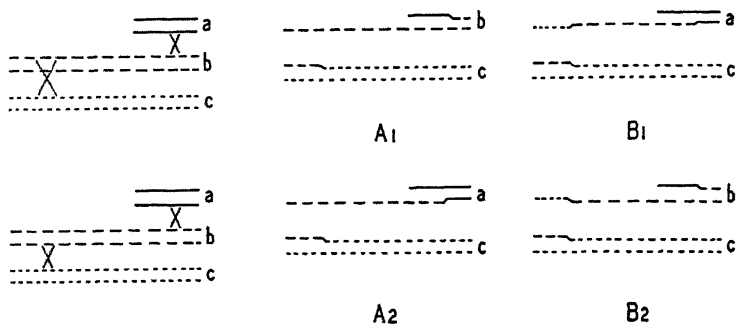


FIGURE 4.—Diagram of double crossing over in a duplication female  $XXX^{pd}$  (carrying free  $X$ 's and fragment), when proximal crossing over occurs between the fragment and an  $X$  ( $X^p/X$ ) and simultaneous distal crossing over between the entire  $X$ 's ( $X/X$ ). It is assumed that crossover fragment and  $X$  pass to opposite poles. The four chromatids that pass to the diplo- $X$  pole after the first (reductional) division are represented by  $A_1$  and  $A_2$  if the  $X$  crossovers go to opposite poles, and by  $A_1$ ,  $A_2$ ,  $B_1$  and  $B_2$  if the  $X$  crossovers segregate at random. After the second (equational) division eggs which have received whole chromatids from a diplo- $X$  pole will contain two  $X$  chromatids each, and if fertilized by  $Y$  sperm produce  $XXY$  females. If  $X$  crossovers always go to opposite poles at the reductional division the  $XXY$  females to be expected are 2 non crossovers: 2 equationals: 0 reciprocals, as shown by the possible combinations of chromatids at  $A_1$  and  $A_2$ . If  $X$  crossovers segregate at random the expected  $XXY$  females are 2 non-crossovers: 4 equationals: 2 reciprocals.

of the four reduced nuclei should be two double crossover  $XX$  females to one equational  $XXY$  female to one non-crossover  $XXY$  female. From four-strand double exchange the expectation is one  $XXY$  female that is equational to one non-crossover (figure 4,  $A_2$ ). Hence the total expectation when  $X$  crossovers pass to opposite poles is 2  $XX$  double crossover females: 2 non-crossover  $XXY$ : 2 equational  $XXY$ . Random disjunction of crossover  $X$  chromatids after three and four strand double exchange includes all the distributions indicated in figure 4. It would result in 2  $XX$  females, from  $X$  eggs containing a double crossover chromosome (derived from the nucleus of the haplo- $X$  pole of the first division) to 8  $XXY$  females from  $XX$  eggs which would be non-crossovers for  $X$ , equationals, and reciprocals in the ratio of 2:4:2.

The experiments yielded small numbers of these infrequent classes and show lack of agreement with either expectation; they show also inconsistencies which do not seem to be entirely due to the small number of observations. It has been noted that only five observed crossovers between the fragment and X ( $X^p/X$ ) are available for determining the expectation of the frequencies of their own and of other classes in experiment 7. Three of the five were double crossovers ( $X^p/X$  and  $X/X$ ). Allocations from ambiguous types make a total of 14  $X^p/X$  crossover females from X-eggs. If the same proportion of these as of the  $X^p/X$  crossovers that were observed (that is about half) may be assumed to be also crossovers between the X's, then 7 at least of 190 observed XXY females would be expected to be equationals. This would be the expectation if the distal crossover X chromatids had disjoined, but twice as many equationals are expected and 7 reciprocals as well if segregation had been random. But of the 105 XXY tested females not one was either a reciprocal or an equational, and of the 85 not tested none was equational whereas only homozygosis for  $y^2$  could not have been detected.

In 5a crossing over of the fragment was not detectable, but among 137 XXY females there were distal crossovers between the X's, table 10 at (b). There were three equationals (one  $y$  and two  $y^2 cv$ , showing crossing over in region 1 or 4 in one chromosome) and there were three reciprocals (involving regions 1, 2, or 4). These occurred among 108 that were tested and represent a total of four equationals (at most) and four reciprocals. The reciprocals show that non-disjunction of crossover X's had taken place in some of the nuclei. Assuming that  $X^p/X$  exchange had occurred with the same frequency as in experiment 7, there is an expectation of eight equationals and four reciprocals from random segregation. A  $y^2 cv v f$  female may have originated from equational non-disjunction. The entire absence of distal crossovers in XXY females in experiment 7 is not explained. Experiment 5a though not in close agreement comes nearer to expectation from random assortment.

In experiment 9, double exchange ( $X^p/X$  and  $X/X$ ) could have been detected in females from XX eggs but none of the 11 XXY females that carried an  $X^p/X$  crossover chromatid was equational for recessive mutant characters.

Thus, the results from different experiments are at variance, for types found in some experiments have not been observed in others in which they would have been detectable. But the results show that almost all of the crossover X's observed in flies from XX eggs in the line of the free fragment may be accounted for as occurring in eggs that have been derived from proximal disjunction of crossover X and fragment as distinguished from reductional non-disjunction of X's; that few  $X/X$  crossovers occur



in flies from  $XXX^{pd}$  gametes; and that whatever X/X exchange was observed in flies from either XX eggs or  $XXX^{pd}$  eggs had occurred in the distal region of the X.

DOBZHANSKY also found that in the lines of X-duplications crossovers are rare in gametes carrying two X's.

It has frequently been observed since first pointed out by BRIDGES (1916) that crossover X's do not occur in XXY females from secondary non-disjunction. GERSHENSON (1935) has found that 0.2 percent of exceptional gametes from  $XX^{InCIB}Y$  females were distal equationals but he found no reciprocal crossovers. This difference is to be expected if crossover chromosomes had disjoined and if proximal disjunction had been determined perhaps by a relation to the Y in a nucleus in which distal crossing over had taken place between the X's. It is consistent with the results of BRIDGES and ANDERSON (1925) in triploids in which they found (distal) equational but not reciprocal crossovers in XX gametes, and with the results of BEADLE (1934) in attached-X triploids in which disjunction is determined by the attachment, and reciprocal crossovers were found with the frequency expected in XX gametes if the second crossover is random.

The evidence from Dp-100 is in agreement with the view that crossover chromatids usually go to opposite poles of the spindle and suggests that in duplication females this holds more consistently for proximal crossovers than it does for distal crossovers, when one of the chromosomes involved has crossed over in a proximal region and disjoined from the third element. The converse question remains whether chromatids that are not crossovers stay together and to this end the distribution of the X homologues in no-exchange tetrads was studied.

The percent of tetrads in which there was no X/X crossing over has been approximately estimated; crossover and non-crossover strands represented by crossover zygotes have been subtracted from the total percents of corresponding strands in the proportions indicated by observed zygotes that were single, double and triple crossovers, on the basis of random exchange between non-sister strands in the four-strand stage. The final residue of non-crossovers is the estimated percent of tetrads containing no crossover strands.

In  $XXX^{pd}$  females (experiment 7) there were 11.0 percent no-exchange tetrads, of which 31.0 percent gave XX- $X^{pd}$  segregation. In experiment 5 there were 13.4 percent no-exchange tetrads and (correcting for 5 percent of X/X crossing over in XXY females) it was found that 35.4 percent of these gave XX- $X^{pd}$  segregation. Thus, in no-exchange tetrads containing the free fragment there is nearly random distribution of the X's and the fragment.

In  $XXX^{pd}$  females (experiment 1) there were 13.3 percent no-exchange

tetrads; the total amount of  $XXX^{pd}$ -O segregation was 0.30 percent which was only 2.3 percent of no-exchange tetrads, showing that disjunction of X from X with a fragment attached was nearly complete irrespective of crossing over. But when a Y chromosome was present there was 19.4 percent of  $XXX^{pd}$ -Y segregation, which was 56 percent of no-exchange tetrads.

#### DISCUSSION

*Crossing over.* The relation of the percents of crossing over between entire X's in the presence of the duplicating fragment to percents from controls in the seven or eight regions that were studied have been plotted against the map of the salivary chromosome (fig. 2), on which are shown the relative positions of mutant genes as located by MACKENSEN (1935) and BRIDGES (1938). The graph shows that in Dp-100 when Y is not present the effect on crossing over is the same (except in one region) whether the fragment is controlled by its own spindle fiber or is attached to one of the entire X's. In both events the greatest reduction is at the spindle fiber end which is the region homologous to the proximal section of the fragment. The curves gradually increase to a maximum in the third region between *cv* and *ct* and then decrease to points somewhat below 1 (control) in the most distal first region which is homologous to the distal section of the fragment.

More detailed examination of the results shows that reduction in X/X crossing over is the same in the lines of the free fragment (1;*f*) and of the attached fragment (1;*r*) in regions 4, 6, 7 and 8; the relations are less regular in region 5. In region 3 the curves for the free lines (1;*f*) reach points higher than 1. The percent of crossing over in experiment 7 alone, or combined with the percents of experiments 3 and 5, is significantly higher than the percent in experiment 1 with the line of attached fragment (1;*r*). In the first region the results are again less different as between the free and the attached fragment.

The differences in region 1 between percents for the duplication and those for the control are so small as not to be significant in any one experiment but are so consistent as to indicate that there is in fact a slight reduction in crossing over.

For further comparison, results from DOBZHANSKY's duplications of the X chromosome and those from experiments with the lines of free and attached fragment of Dp-100 are presented graphically in figure 5.

DOBZHANSKY (1934) has shown that in the duplications studied by him frequency of exchange between the entire X chromosomes is reduced in the presence of the duplication fragments, that the relatively greatest reduction is observed in the regions homologous to the fragments and in



Dp-138 extends from the spindle attachment to a point distal to the locus of rudimentary and contains therefore an active section of X from *r* to *Bx* not included in Dp-100. The reduction in crossing over in the *f-car* region (7th) in Dp-100 is as great as in Dp-138. There was no control for Dp-138 in region 8, but using the control for experiment 7 (which is the same in regions 6 and 7 as the control for Dp-138) crossing over appears to be reduced more than in Dp-100 in region 8. Thus the effect of the proximal component of the fragment of Dp-100 is consistent with the results from the other duplications, including the reduction found in region 7 in duplications composed largely of a distal section.

But when the distal effect of Dp-100 is compared with that of largely distal duplications the result is quite different. The slight reduction indicated as probably occurring in the region homologous to the distal section of Dp-100 is by no means comparable, in relation to lengths of fragments, to the reductions found in the other "distal" duplications (only Dp-102 gives an inconsistent result in the 1st region and in the 7th but not in the 2nd which is homologous to the fragment). The distal effect of Dp-100 is like that of the much shorter Dp-101 and the effect is much less than that of the somewhat shorter Dp-134.

It is evident that the position of the components of the fragment may be a very important factor in their effects. In this connection it may be noted that the long proximal Dp-138 has a much greater effect on X/X crossing over than has the long distal section of Dp-105. Dp-100 has, in proportion to the length of its proximal component, an effect comparable to that of the simple proximal Dp-138, in the region of the X's homologous to the proximal component, and the distal effect also is not very different in spite of the presence of the distal component in Dp-100. Thus the results suggest an advantage in pairing at the proximal end of the chromosome over pairing at the distal end.

DUBININ et al (1935) showed that, in the salivary glands, short proximal sections of chromosomes pair rather than much longer distal sections.

PAINTER (1934 and 1935) had found the same kind of pairing in the salivary chromosomes in Mottled-5 and other translocations of the fourth chromosome. He found that in a heterozygote the normal fourth chromosome tends to pair with a very small segment of the fourth at the spindle fiber end rather than with the segment comprising almost the entire fourth chromosome attached to the X near its distal end, or to a point on a large autosome far removed from the spindle fiber attachment. He points out the importance of the telophase orientation in pairing and the possible application in the understanding of pairing at meiosis in forms in which there are aberrant elements.

DOBZHANSKY (1936) has shown that the chromosome pattern tends to

persist through the interphase in spermatogonial divisions in *Drosophila pseudoobscura* and emphasizes the importance of taking into account the telophase orientation and spatial distribution of chromosomes in the study of crossing over variations in chromosomal aberrations.

With the telophase orientation as the key to the situation, the proximal component of the compound duplication is expected to be, at the time of pairing, in a position similar to that of a simple proximal fragment, and the proximal effects of Dp-100 are in fact comparable to the effects of the proximal Dp-138. In considering the effect of the distal component its position in the nucleus is a factor to be taken into account. In relatively simple "distal" duplications containing more or less of the inert region, the position of the proximal end of the distal fragment at telophase is near to the spindle fiber attachment or near to the locus of *bb* at the farthest; in Dp-100 its position is determined by its attachment to the distal end of the proximal fragment (near the locus of *fu*) which, due to the pairing of the proximal component, may be a relatively fixed point at about the middle of the X's. In this position the distal fragment has been shown to have scarcely any effect on the crossing over between the X's at their distal ends, whereas a shorter fragment in Dp-134 proximally attached to its own spindle fiber attachment and not including the locus of *bb* has a marked effect. In fact, in proportion to the lengths of the distal sections, Dp-134 has a relatively greater effect in the distal homologous region than have Dp-101 and Dp-105, both of which include the locus of *bb*; and Dp-134 has a lesser effect than either in region 7 (the most proximal region that was observed). Proximal sections are effective in proportion to their lengths; distal sections, attached to a proximal section, are effective directly in proportion to their lengths and inversely to the distances by which they are removed from the spindle fiber attachment.

The fact that the effect of the distal section of a compound duplication is conditioned by the extent of the proximal section is evidence that telophase orientation is important, and is consistent also with MATHER's conclusion that in *D. melanogaster* pairing takes place first near the spindle attachment. His conclusion is based on a study of the relation of points of crossing over to the spindle attachment and to each other. It is also consistent with the relation found in inversions by STONE and THOMAS (1935) in agreement with observations of STURTEVANT and BEADLE (1936) who state "that an inversion is more effective in suppressing crossing over in segments distal to itself than in proximal segments." Since reduction in crossing over between the X's is the same whether the fragment is free or is attached, the telophase orientation appears to be in this regard as effective as a permanent attachment.

It has been seen that the effects of the compound Dp-100 are similar to

those of the proximal Dp-138 in the regions that are homologous to both components of Dp-100; in the intermediate regions there are two differences. Proceeding distally from the region homologous to the fragment, in Dp-138 crossing over rises abruptly to the same frequency as the control in the region beyond the fragment whereas in Dp-100 the ratios increase gradually and in region 3 crossing over is more frequent than in the control, a peak only slightly shown in Dp-138 and not in the "distal" duplications. It is of interest to note that the peak occurs in a region which has a high coefficient of crossing over, the highest in fact of all regions of the complete chromosome complement (MORGAN, BRIDGES and SCHULTZ 1937).

The effects on crossing over in regions not homologous to the components of the fragment must in their turn be dependent upon spatial relations correlated with the peculiar pairing relations of the homologous regions. Furthermore the whole chromosome complement is involved in disturbances of crossing over in a given region, as shown by interarm effects and by interchromosomal effects (DOBZHANSKY and STURTEVANT 1931, DOBZHANSKY 1930, 1933, MORGAN, BRIDGES and SCHULTZ 1930, 1932, 1933, 1935, STEINBERG 1936 and MACKNIGHT 1937).

DOBZHANSKY has shown that the frequency of exchange between the fragment and an entire X is not at all commensurate with the reduction in crossing over between the X's in the homologous region. The two are however positively correlated and are correspondingly correlated with the length and position of the fragment.

Crossovers between a free fragment and one of two attached X's were observed by DOBZHANSKY in two duplications. In the long proximal Dp-138 he found crossing over between the proximal fragment and an X in 0.7 percent of gametes, and in the distal Dp-105 crossing over was apparently much less frequent. This relation corresponds to a greater reduction in crossing over between the X's in the regions homologous to the fragment in Dp-138 than in Dp-105, the ratios of percents of X/X crossovers in the duplications to control being 0.11:1 and 0.34:1 respectively (the smaller ratio representing the greater reduction).

The values for the duplicated regions of Dp-100 are consistent with these. For the proximal region, the values are nearly the same as those for Dp-138 and are intermediate between those for Dp-138 and Dp-105. In Dp (1;f)100, it is estimated that the frequency of  $X^p/X$  crossing over when the fragment is free is 0.5 percent and the ratio of X/X exchange to control (in the 7th and 8th regions) is 0.13:1. Exchange between the distal component and an X ( $X^d/X$ ) takes place rarely and corresponds to a very slight distal reduction in X/X exchange, represented by the ratio 0.85:1.

Although the frequency of crossing over of the fragment increases as the reduction in crossing over between the X's increases,  $X^{Dp}/X$  crossing over is obviously only a small fraction of the total crossing over in the homologous regions, even with the longest of the fragments that have been studied. If exchange were random among the three homologues the expected ratio of  $X^{Dp}/X$  to  $X/X$  crossing over would be 2:1; in Dp-105, Dp(1;f)100 and Dp-138 the ratios are less than 0.13:1, 0.30:1 and 0.50:1 respectively. The three duplications are mentioned in the order of increase in  $X^p/X$  crossing over, and the ratios show further that increase of frequency of crossing over involving the fragment is positively correlated with increase in its proportion of total crossing over in the homologous region.

PHILIP (1934) studied crossing over in a duplication, known as Dp-B<sup>S</sup>, in which the fragment is a proximal section of the X chromosome, (including the locus of *B* to the spindle-fiber attachment) to which most of the fourth chromosome is attached. Corrected data from females give 0.14 percent of crossing over between attached X's and the free fragment. The value is low compared to 0.7 percent for the somewhat longer proximal Dp-138 in the same form ( $XXX^p$ ). When the fragment of Dp-B<sup>S</sup> was attached to an X, the corrected data from females give 0.2 percent of  $X^p/X$  crossing over. This does not show the decrease in  $X^p/X$  crossing over, when the fragment is attached, which was found in Dp-100. An important factor in Dp-B<sup>S</sup> is the attachment of the fourth chromosome at the distal end of the fragment which originated in a I, IV translocation.

DOBZHANSKY has compared two translocations T-3 and T-7 with the duplications derived from them and has reached conclusions that may be further analyzed. In each of the duplications the distal fragment of the X is attached at the end of an arm of one of the large autosomes and may pair with the two X chromosomes. In the corresponding translocations the only exchange in the distal region of the X is that which occurs between the fragment carried by the autosome and the single whole X. In a translocation, synapsis of the fragment is the condition for exchange in the region homologous to the fragment; in the corresponding region in a duplication the presence of the fragment is a factor in reduction of crossing over between the entire X's and of total crossing over in the region; the pairing of the autosomes involved is a factor affecting synapsis of the X's in both forms. DOBZHANSKY states that as a rule crossing over is more reduced in translocations than in duplications derived from them. His data show further that this relation holds for T-3 and T-7 only for the region homologous to the fragment, where crossing over between the fragment and the single entire X in the translocations was less frequent

than crossing over between the entire X's in the corresponding duplications.

The regions not homologous to the fragment in T-3 and T-7 were present in the duplications in two chromosomes that were entire X's, and they were present in the translocations in one entire X and in a proximal fragment comprising a large portion of the X (from the spindle fiber attachment to a point proximal to ruby). The total frequency of cross-overs in these regions between the partial X and the entire X in the translocations was the same as between the entire X's, in the corresponding duplications. The nature of the effect of a translocation on crossing over is perhaps more clearly shown in ANDERSON'S (1929) line of high non-disjunction. The X was broken near the locus of vermilion into two long segments and the distal fragment translocated onto chromosome III. The greatest reduction in crossing over between the X's was in the region of the break and the reduction decreased toward both ends of the X.

DOBZHANSKY'S data for T-3 show the relative frequencies with which the distal fragment crosses over when present in the heterozygous translocation and in the duplication. There were 0.42 percent crossovers between the distal fragment and the one entire X in the first region in the translocation (and some additional crossing over involving the fragment since it overlapped about half of the second region). In the duplication there were only 0.21 percent crossovers between the fragment and both of the X's.

Although the fragment in the duplication from T-3 was about three-fourths of the length of the distal section of Dp-105 and the reduction in crossing over between the X's was correspondingly not so great, still in Dp-T-3 the ratio of  $X^d/X$  to  $X/X$  crossing over (in the homologous region) was more than 0.13:1 as compared with a ratio much less than 0.12:1 in Dp-105 when the fragment was present with attached X's. This suggests that the fragment of Dp-T-3, in its position at the end of a large autosome, may have an advantage in crossing over, over a distal fragment attached to a short proximal region, without increase in its effect on crossing over between the entire X's. A similar advantage to a fragment in a free state rather than attached to an X has been shown in  $X^p$  of Dp-100. In Dp-105 the ratio of  $X^d/X$  to  $X/X$  crossing over was 0.02:1 when the fragment was attached to another X, and less than 0.12:1 when free and the X's were attached to each other.

DOBZHANSKY (1934) has pointed out that the effect of a fragment on crossing over in regions homologous to itself is opposite to that of a third X in a triploid. Total crossing over among X's in triploids is higher than in diploids, especially in the most proximal and distal regions (BEADLE 1934). DOBZHANSKY'S study and the present study of X-duplications show



that the longer the fragment (in a given position) the greater the reduction in the total crossing over among the three units of the system and the greater the proportion of crossing over of the fragment. The limiting hypothetical situation is a system of three entire X's (the superfemale condition) in which crossing over should occur infrequently, and at random if at all.

BEADLE and EPHRUSSI (1937) have recently found that the offspring from the ovary of a superfemale transplanted into a normal female show almost no crossing over among the three X's. But the authors' analysis of this and of other irregularities leads them to conclude that the chromosome unbalance characteristic of super females has a specific effect on the meiotic mechanism of all of the chromosomes.

In the conditions prevailing in triploids, in which crossing over is increased, REDFIELD (1930) has found that when crossover reducers are present in one of three homologues of a triploid "the remaining two chromosomes cross over as they do in triploids and not as they do in diploids."

#### DISJUNCTION

The diagram used by STURTEVANT to show the disjunction relations that hold among three fourth chromosomes has been found convenient for representing the types of segregation of the sex chromosome homologues under discussion. STURTEVANT, in estimating the frequency with which a fragment of the X chromosome will segregate with attached X's rather than with Y (in  $XXX^{pd}Y$  females) applied his results obtained for the fourth chromosome, but it appears from the present study that the relations of the chromosomes at disjunction established for the fourth chromosomes do not hold for the sex chromosomes. By use of the diagram and of certain definitions, STURTEVANT illustrates a constant relation of disjunction between any two given fourth chromosomes of his series. He found that his results were verifiable if he assumed that, when two of three chromosomes go to opposite poles of the meiotic spindle, the proportion in which one of them goes to the diploid pole is a property of those two chromosomes and is independent of the nature of the third opposing chromosome.

This is found to be not true for X and Y when the third chromosome is another normal X or one which is altered in one way or another. For example, if an X and a Y chromosome are present with an X containing the inversion  $dl-49$  then, when X separates from Y, the proportion of times in which X segregates with  $X^{dl-49}$  is the frequency with which X passes to the diploid pole divided by the sum of the frequencies with which it passes to the diploid and to the haploid pole. Using this formula, which

gives a constant value for any two fourth chromosomes, it is found that when X separates from Y the proportion in which it goes to the diploid pole is not constant when the third opposing chromosome is changed. The different values are 0.113, 0.726, 0.77 and 0.365 when the opposing elements are respectively normal X, In-CIB, In-dl-49 and an X with the fragment of Dp-100 attached to it. Obviously there are differences in the four examples in the crossover relations between the X (and the Y) and the various opposing X's. The effect of crossing over on disjunction probably enters into the problem. In XXY almost normal crossing over takes place between the X's. In XX<sup>In</sup>Y there is a reduction in crossing over due to the inversion in the opposing X. In the duplication an agent reducing crossing over is the fragment introduced by the third element, which is the fragment and X attached to each other. There may be other factors affecting disjunction due to the attached fragment. At the time of disjunction the proportion of tetrads containing only non-crossover chromatids varies in the four combinations.

STURTEVANT and BEADLE (1936) have studied the distribution of homologues in females heterozygous for X inversions and have found that the X's of nearly all tetrads in which no exchange has taken place segregate together when a Y chromosome is present. In XX females heterozygous for In-dl-49, in which exchange takes place in only about 30 percent of tetrads, disjunction of the X's occurs regularly. But when a Y chromosome is present, they have found that in 90 percent of no-exchange tetrads the X's pass together to one pole away from the Y.

In the line of attached fragment, Dp (1;1)100 (without Y), disjunction of the X's is nearly complete (there are about 0.30 percent of exceptions) although there are 13.3 percent no-exchange tetrads. The pairing relations concern three elements which in disjunction are reduced to two units. In the presence of Y there is 19 percent of non-disjunction of X's and the frequency with which the two X's segregate together in no exchange tetrads is 56 percent. Disjunction of the fragment is determined by its attachment to an X, and non-disjunction of X's in no-exchange tetrads is also in this combination increased by the presence of Y.

It has been seen that in Dp(1;f)100 with two normal X's and a free fragment 31 percent of no-exchange tetrads give non-disjunction of X's. This results in a total percent of exceptions which is nearly the same as the percent of secondary exceptions from XXY females in which crossing over is normal, but the analysis shows that the conditions leading to approximately the same result are different. The percent of exchange tetrads is greatly reduced by the presence of the fragment and disjunction in no-exchange tetrads is nearly random in the duplication female.

Observations have been made by STURTEVANT and BEADLE on disjunc-

tion in systems of three homologues when one of the X's carries an inversion and the third element is a Y or a short fragment, the length of the fragment being varied.

The Y chromosome has been found by them (STURTEVANT and BEADLE 1936) to be but slightly concerned with reduction in crossing over between the X's (for example in In-dl-49). They refer to a difference between the effect of Y and of a small fragment of X on the percent of exceptions in females heterozygous for In-CIB. Unpublished data from experiments done separately by the two authors show that the frequency of exceptional zygotes from CIB/+ is almost 0 (as in XX), from CIB/+Y it is 40 percent and from CIB/+X<sup>Dp</sup> it is 5-10 percent, corresponding respectively to 57 percent and 9-18 percent of segregation of the X's to the same pole.

In XXX<sup>Dp</sup> females (Dp(1;f)100), non-disjunction of X's is nearly random in no-exchange tetrads, whereas in XX<sup>d1-49</sup>Y females, STURTEVANT and BEADLE found nearly complete non-disjunction of X's (90 percent) in no-exchange tetrads. If 57 percent of XX<sup>1a</sup>-Y segregation represents 90 percent of no-exchange tetrads in CIB and if fragments of X produce an effect similar to that of the fragment in Dp(1;f)100 then about one-third of 57 percent or 19 percent of XX<sup>CIB</sup>-X<sup>Dp</sup> segregation might be expected from duplication females heterozygous for CIB. The percent would vary with the length of the fragment since reduction in the amount of crossing over and probably the frequency of no-exchange tetrads are correlated with the length of the fragment.

The effect on disjunction of a fragment of X with the long arm of Y attached to it, described by PHILIP (1934), is in one combination of X homologues comparable to the effect of a Y chromosome. She found that in attached-X females carrying the proximal fragment of Dp-B<sup>S</sup> the frequency of XXX<sup>Dp</sup>-O segregation was 8.7 percent; but, when the long arm of Y was attached to the fragment, there was nearly complete disjunction of this new unit from the attached X's (the frequency of XXX<sup>Dp</sup>Y'-O segregation was only 0.33 percent). Complete disjunction occurs also in XXY females.

The frequency of XXX<sup>Dp</sup>-O segregation in the original female of Dp-100 was 25.8 percent and when a Y was present the frequency of segregation of the fragment with the attached X's away from Y (XXX<sup>Dp</sup>-Y) was 75.9 percent. Comparison of duplications of DOBZHANSKY's study shows opposite correlations of the frequencies of the two types of segregation with the length of the proximal section of the fragment. DOBZHANSKY observed a negative correlation between the length of fragment and the frequency of XXX<sup>Dp</sup>-O segregation. In the duplications studied by him that contained very short fragments of the distal region of X and more

or less of the inert region,  $\text{XXX}^{\text{Dp}}$ -O segregation was less frequent when the locus of  $bb$  was included, except in Dp-102 which has already been referred to as exceptional in crossover relations. The frequencies were 10-19 percent when the locus of  $bb$  was present and 24-27 percent in its absence. When a Y chromosome was present with the same fragments non-disjunction of the attached X's and fragment was increased but in such proportions that  $\text{XXX}^{\text{Dp}}$ -Y segregation was more frequent when the locus of  $bb$  was included than when it was not present; non-disjunction was over 50 percent when the fragment covered  $bb$  and less than 50 percent when it was shorter. (One exception (Dp-106) which seemed when tested not to contain the locus of  $bb$  nevertheless gave frequencies of the two segregations similar to those of the other duplications covering  $y$  *svr* and  $bb$ ; SIVERTZEV-DOBZHANSKY and DOBZHANSKY (1933) state that the tests were not altogether conclusive.) The relation of crossovers to disjunction in these combinations of attached-X's and fragment is not known.

A constant property of the Y chromosome is the effect of increasing non-disjunction of two other units of X homologues in combinations that have been described by others and in this study; non-disjunction is increased when Y is present with XX,  $\text{XX}^{\text{In}}$ ,  $\text{XXX}^{\text{Dp}}$  and  $\text{XXX}^{\text{Pd}}$ .

#### ACKNOWLEDGMENTS

I gratefully acknowledge my indebtedness to Dr. JACK SCHULTZ, Dr. A. H. STURTEVANT, and Dr. G. W. BEADLE for encouragement and assistance and for the use of unpublished data, and to Dr. TH. DOBZHANSKY, Dr. A. WEINSTEIN and especially to Dr. C. B. BRIDGES for aid in preparation and revision of the manuscript.

#### SUMMARY

1. The duplicating fragment of Dp-100 is an X chromosome deficient for the middle section. The fragment contains a distal component extending from the distal end of the chromosome (yellow) to a point between the loci of prune and white, and a proximal component extending from a point between the loci of Beadex and fused to the spindle fiber end. The fragment therefore contains the inert region, the section homologous to Y, and some of the active region of the middle and distal end of the X.

2. The fragment produces characteristic somatic effects and lowers viability in females. It is usually lethal in males. It may sometimes produce a fertile intersex when present with two entire X's in triploids (DOBZHANSKY and SCHULTZ 1931).

3. The homologues of X that are involved have been obtained in the combinations: (a)  $\text{XXX}^{\text{Pd}}$ , (b)  $\text{XXX}^{\text{Pd}}$ Y, (c)  $\text{XXX}^{\text{Dp}}$ , (d)  $\text{XXX}^{\text{Pd}}$ Y and (e)  $\text{XXX}^{\text{Pd}}$  (fig. 1).

4. Crossing over has been studied in the last three combinations (in which the X's are not attached to each other) in eight regions from yellow to bobbed, and a marked reduction in crossing over between the X's has been observed in the region homologous to the proximal component of the fragment (fig. 2). There is also a marked reduction in total crossing over in that region.

5. In the two lines with separate X's without Y, (e) and (c), reduction in crossing over between the entire X's is the same (except in region 3) whether the fragment is free (e) or is attached to one of the X's (c).

6. In lines (e) and (c) crossing over between X's is reduced to about one-tenth of the frequency of the control in the eighth region (nearest to the spindle fiber attachment). Toward the distal end of the chromosome, the curve representing ratios of duplication to control crossing over rises gradually to a maximum higher than control in the third region between *av* and *ct*; it then falls but only to about 0.8 in the first region which is in part homologous to the distal component of the fragment ( $X^d$ ).

7. When a Y chromosome is present and the fragment is attached to an X (d), crossing over is slightly lower throughout the length of the chromosome and still further reduced in the eighth region.

8. Crossing over takes place between the distal component of the fragment ( $X^d$ ) and an entire X, with a very low frequency (0.01 to 0.04 percent).

9. Crossing over occurred between the proximal component of the fragment ( $X^p$ ) and X. In the line of free X's and fragment (e) the frequency was estimated by allocations of ambiguous types from X eggs in experiment 7, or by marking the X's with *bb'* which reduces exchange. The chromatids that are  $X^p/X$  crossovers seem to occur at least as frequently in XX as in X eggs. The frequency of  $X^p/X$  crossing over appears to be about 0.5 percent in the  $XXX^{pd}$  line (e).

10. Corrected frequency of crossing over between  $X^p$  and X in the line in which the fragment is attached to an X (c), as estimated from exchange between  $X^p$  and the free X, and diagonal exchange between  $X^p$  and attached X, was less than 0.1 percent.

11. When a Y was also present the frequency was 0.2 to 0.3 percent.

12. In the line of free X's and free fragment (e) crossovers between X's were rarely observed in zygotes from XX gametes known to be not crossovers between the proximal component of the fragment ( $X^p$ ) and an X (1 equational in region 5 among 594 females). In experiment 7 half of the  $X^p/X$  crossovers from X eggs were also distal crossovers between the X's; but no X/X crossovers were observed in any of the females from XX eggs. In experiment 5a, a small percentage of both reciprocal and equational distal crossovers was observed in flies from XX eggs, showing that distal

X/X crossovers do not always disjoin. The frequency was not higher than was to be expected if the X/X crossovers were also  $X^P/X$  crossovers, which were not detectable.

13. In the lines with attached fragment, with a Y (d) or without (c), five distal equationals were observed among 1204 exceptional females. About half of the  $X^P/X$  crossovers which were recovered in attached-X females from XX eggs were also X/X crossovers in distal regions.

14. Crossing over between Y and the other X homologues was not studied and was observed only in the  $XXX^{Pd}Y$  line (b).

15. Disjunction of the different partially homologous elements has been studied in the five lines of Dp-100:

a. The frequency of  $XXX^{Pd}-O$  type of segregation in the original female (a) was 25.8 percent.

b. With Y added, in the  $XXX^{Pd}Y$  line (b), there was 75.9 percent of  $XXX^{Pd}-Y$  type of segregation, that is, more than the frequencies of both of the other types of segregation. This shows that in this combination of X homologues disjunction is not dependent on random distribution of one of the elements after separation of the other two. The result is similar to those obtained by BEADLE and STURTEVANT (1936) with X inversions, by STURTEVANT (1936) with triplo-IV, and by DOBZHANSKY (1934) and STURTEVANT (1936) with X duplications.

c. The frequency of segregation of two X's together ( $XX-X^{Pd}$ ) in experiment 7, with the line of free X's and free fragment (e), was 3.4 percent, that is, about one-third of the estimated percent of no-exchange tetrads, a nearly random distribution in no-exchange tetrads of the three homologues. Segregation in this combination is markedly different from that in  $XX^{In}Y$  females, heterozygous for the dl-49 inversion, in which the X's segregate together in 90 percent of no-exchange tetrads (STURTEVANT and BEADLE 1936). These comparisons in connection with others show a distinct difference between the effects of Y and of even small fragments of X on disjunction of X's. PHILIP (1934) found that an element composed of the long arm of Y attached to a proximal fragment (extending to the locus of *B*) disjoins completely from attached X's. The effect is the same as that of Y in  $XXY$  females but different from that of the fragment of Dp-100 (15a above) and of other duplications (DOBZHANSKY 1934).

d. Disjunction of the two X's, when the fragment is attached to one of the X's, in the  $XXX^{Pd}$  line (c) of Dp-100 is almost complete as in  $XX$  or  $XX^{In}$  females; the frequency of  $XXX^{Pd}-O$  segregation was 0.3 percent which is about the same as in  $XX$  controls, and is only 2.3 percent of the frequency of no-exchange tetrads.

e. The presence of a Y with free X's and attached fragment (d) increases

segregation together of the X's ( $\text{XXX}^{\text{pd}}\text{-Y}$ ) to about 19.0 percent. This is about 56 percent of the frequency of no-exchange tetrads.

16. The reductions in crossing over between X's in the region homologous to the proximal component of the fragment in Dp-100 and in the proximal Dp-138 studied by DOBZHANSKY (1934) are proportional to the respective lengths of the duplicating sections of chromosomes (shown graphically in figure 2). This is in agreement with the correlations shown by DOBZHANSKY in other duplications.

17. The slight reduction in crossing over between the X's in Dp-100, in the region homologous to the distal component of the fragment, is not as great, relative to the lengths of fragments, as the reductions found by DOBZHANSKY in distal duplications of varying lengths having their own spindle fibers and containing more or less of the inert region. The reduction in the distal region in Dp-100 is comparable to that found in a much shorter "distal" duplication (Dp-101), which includes the locus of *bb*.

18. The effect of Dp-100 is throughout the whole X very similar to the effect of the simple proximal Dp-138, some differences appearing in the regions not homologous to the fragments.

19. The results show that the proximal component of the fragment has more effect than the distal on crossing over of the X's in homologous regions relatively to their lengths. Other comparisons show correlation between the extent of effects of fragments and their position.

20. In the proximal region of Dp-100, the proportion of  $\text{X}^{\text{p}}/\text{X}$  crossing over to  $\text{X}/\text{X}$  crossing over, in experiment 7 with the line of free X's and free fragment (e), is about 0.30:1 whereas if exchange were random the ratio would be 2:1. Other duplications show a similar relation.

21. Comparison of Dp-100 with DOBZHANSKY's proximal Dp-138 and mostly distal Dp-105 shows that increase in reduction of crossing over in the region homologous to the fragment is positively correlated with increase in reduction of total crossing over in that region, and with increase in the proportion of the total that involves the fragment. In some conditions, there appear to be factors that give an advantage in crossing over to the fragment without changing the extent of its effect on crossing over of the X's.

22. The persistence of the telophase relations of the chromosomes whereby the regions of spindle-fiber attachments are closely associated before the first meiotic division and whereby new relations are established in aberrations has been shown by DOBZHANSKY (1936) to be an important consideration in the study of crossing over in aberrations. By a comparison with other duplications a proportional effect on crossing over of the proximal component of Dp-100 is shown. The position of a distal section in a duplication is determined by its attachment to a long or short proximal

component or to another chromosome. On the relatively long proximal section of Dp-100 the distal component has less effect on X/X crossing over than have comparable distal fragments when attached to short proximal sections containing more or less of the inert region only, or when carried at the ends of the long autosomes.

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## APPENDIX

Tables 6-10. For each type of crossover the complementary class first recorded in the tables is that containing the distal end of the first entire

TABLE 6

Experiment 3. Offspring of  $Dp(1:f)/y^2 cv v f/y ec cl^8 s car \text{ } \varnothing$  by  $yXg \text{ } \sigma^7$ .

| TYPE | NON-Dp<br>( $\sigma^7 \varnothing$ ) |     | Dp<br>( $\varnothing \varnothing$ ) |     | CONTROL<br>( $\sigma^7 \varnothing$ ) |     | TYPE    | NON-Dp<br>( $\sigma^7 \varnothing$ ) |      | Dp<br>( $\varnothing \varnothing$ ) |     | CONTROL<br>( $\sigma^7 \varnothing$ ) |     |
|------|--------------------------------------|-----|-------------------------------------|-----|---------------------------------------|-----|---------|--------------------------------------|------|-------------------------------------|-----|---------------------------------------|-----|
| 0    | 512                                  | 567 | 238                                 | 146 | 295                                   | 267 | 3, 5    | 1                                    | 1    | —                                   | 1   | 4                                     | 2   |
| 1    | 51                                   | 65  | (with 0)                            |     | 38                                    | 31  | 3, 6    | 3                                    | 4    | —                                   | 2   | 3                                     | 7   |
| 2    | 87                                   | 74  | 10                                  | 15  | 44                                    | 48  | 3, 7    | —                                    | —    | (with 3)                            |     | 2                                     | 1   |
| 3    | 88                                   | 84  | 6                                   | 28  | 38                                    | 61  | 4, 5    | 1                                    | 3    | 2                                   | 1   | 4                                     | 5   |
| 4    | 119                                  | 117 | 31                                  | 41  | 81                                    | 74  | 4, 6    | 4                                    | 4    | —                                   | —   | 4                                     | 5   |
| 5    | 65                                   | 65  | 7                                   | 27  | 48                                    | 42  | 4, 7    | —                                    | 1    | (with 4)                            |     | 3                                     | 6   |
| 6    | 45                                   | 78  | 17                                  | 8   | 60                                    | 62  | 5, 6    | 1                                    | —    | —                                   | —   | —                                     | —   |
| 7    | 8                                    | 10  | (with 0)                            |     | 26                                    | 22  | 5, 7    | 2                                    | 1    | (with 5)                            |     | 1                                     | 3   |
| 1, 2 | 2                                    | —   | (with 2)                            |     | —                                     | 1   | 6, 7    | 1                                    | 1    | (with 6)                            |     | 1                                     | —   |
| 1, 3 | 3                                    | 4   | (with 3)                            |     | —                                     | —   | 1, 2, 7 | —                                    | —    | (with 2)                            |     | 1                                     | —   |
| 1, 4 | 5                                    | 5   | (with 4)                            |     | 7                                     | 5   | 1, 4, 5 | —                                    | 1    | —                                   | —   | —                                     | —   |
| 1, 5 | 6                                    | 7   | (with 5)                            |     | —                                     | 5   | 1, 4, 6 | —                                    | —    | —                                   | —   | 1                                     | —   |
| 1, 6 | 4                                    | 7   | (with 6)                            |     | 7                                     | 6   | 1, 5, 6 | 1                                    | —    | —                                   | —   | —                                     | —   |
| 1, 7 | —                                    | 1   | (with 0)                            |     | 5                                     | 1   | 1, 5, 7 | —                                    | —    | (with 5)                            |     | 1                                     | —   |
| 2, 3 | 1                                    | —   | —                                   | —   | —                                     | —   | 1, 6, 7 | —                                    | —    | (with 6)                            |     | 1                                     | —   |
| 2, 4 | 4                                    | 8   | —                                   | 2   | 6                                     | 5   | 2, 3, 6 | —                                    | —    | —                                   | —   | —                                     | 1   |
| 2, 5 | 2                                    | 5   | 1                                   | —   | 3                                     | 3   | 2, 4, 7 | —                                    | —    | (with 2, 4)                         |     | 1                                     | —   |
| 2, 6 | 7                                    | 5   | —                                   | 2   | 16                                    | 10  | 3, 5, 7 | 1                                    | —    | (with 3, 5)                         |     | —                                     | —   |
| 2, 7 | 2                                    | —   | (with 2)                            |     | 5                                     | 1   |         | 1027                                 | 1118 | 312                                 | 274 | 708                                   | 676 |
| 3, 4 | 1                                    | —   | —                                   | 1   | 2                                     | 2   | Total   | 2145*                                |      | 586                                 |     | 1384                                  |     |

\* Class (a) from X-XXX<sup>rd</sup> segregation. Other classes: (b) 40 XXV females not equational (2 XXV females in control); (c) X<sup>p</sup>/X crossovers included in (a) and (b); (d) X<sup>d</sup>/X crossovers none.

Quota of chromatids used in determining percent of crossing over: 2145 from males and females of class (a), 80 represented by class (b).

chromosome to be described, namely, containing  $y^2$ , while the second entry is of the complementary class beginning with  $y$ . Numbering of regions follows the scheme:  $y^2(y)1\ ec\ 2\ cv\ 3\ ct\ 4\ v\ 5\ s\ 6\ f(B)\ 7\ car\ 8\ bb(bb^1)$  illustrated in figure 1 e. The tester male used in test crosses known as  $yX_9$  ( $y\ ec\ cv\ ct^6\ v\ s^2\ car\ f\ bb^1$ ) carries a marker for every region.

Although in the actual experiments females and males were classified and totalled separately they have been combined in the following tables since careful inspection showed close agreement throughout. The duplication classes include only females, and since their viability is erratic, complementary crossovers may differ widely in value; they were not used in determining percent of crossing over.

TABLE 7

*Experiment 5. Offspring of  $Dp(1;f)/y^2\ cv\ v\ f/y\ ec\ ct^6\ s\ car\ \varnothing\ by\ f\ B\ \sigma^7$ , and from  $XXY$  daughters  $by\ f\ B\ \sigma^7$ .*

| TYPE | XXX <sup>pd</sup><br>NON-Dp<br>( $\sigma^7\sigma^7$ ) |     | XX<br>CONTROL<br>( $\sigma^7\sigma^7$ ) |    | XXY |     | TYPE    | XXX <sup>pd</sup><br>NON-Dp<br>( $\sigma^7\sigma^7$ ) |     | XX<br>CONTROL<br>( $\sigma^7\sigma^7$ ) |     | XXY  |     |
|------|---|-----|---|----|-----|-----|---------|---|-----|---|-----|------|-----|
| 0    | 386   | 379 | 129                                     | 73 | 430 | 304 | 3, 4    | 4   | —   | —                                       | —   | 1    | —   |
| 1    | 44  | 31  | 11                                      | 12 | 23  | 38  | 3, 5    | 3   | —   | —                                       | —   | 6    | 5   |
| 2    | 63  | 49  | 19                                      | 8  | 72  | 56  | 3, 6    | 3   | 2   | 2                                       | —   | 3    | 9   |
| 3    | 53  | 78  | 12                                      | 11 | 49  | 56  | 3, 7    | —   | 3   | —                                       | 1   | 4    | 2   |
| 4    | 105   | 98  | 25                                      | 30 | 114 | 104 | 4, 5    | 2   | —   | 3                                       | —   | 3    | 6   |
| 5    | 50  | 34  | 23                                      | 8  | 65  | 61  | 4, 6    | 3   | 1   | 2                                       | —   | 10   | 10  |
| 6    | 33  | 36  | 19                                      | 16 | 53  | 63  | 4, 7    | —   | 1   | 6                                       | 1   | 2    | 9   |
| 7    | 8   | 5   | 6                                       | 7  | 29  | 21  | 5, 6    | —   | —   | —                                       | 1   | —    | —   |
| 1, 2 | —   | —   | —                                       | —  | —   | 1   | 5, 7    | —   | 1   | 1                                       | —   | —    | 2   |
| 1, 3 | 1   | —   | —                                       | —  | 1   | —   | 6, 7    | —   | —   | —                                       | —   | —    | —   |
| 1, 4 | 4   | 1   | 3                                       | 2  | 3   | 4   | 1, 3, 7 | —   | —   | —                                       | —   | —    | 1   |
| 1, 5 | 5   | 2   | 1                                       | —  | 4   | 2   | 1, 4, 5 | —   | —   | —                                       | —   | —    | 1   |
| 1, 6 | 5   | 4   | 1                                       | 3  | 5   | 9   | 2, 4, 6 | —   | —   | —                                       | —   | —    | 2   |
| 1, 7 | 1   | 2   | 2                                       | 1  | 2   | 3   | 3, 4, 6 | —   | —   | —                                       | —   | —    | 1   |
| 2, 4 | 5   | 3   | 3                                       | 1  | 6   | 4   | 4, 5, 7 | —   | —   | 2                                       | —   | —    | —   |
| 2, 5 | 2   | 2   | 4                                       | —  | 12  | 7   |         |   |     |   |     |      |     |
| 2, 6 | 4   | 2   | 3                                       | 1  | 8   | 12  |         | 784   | 734 | 278                                     | 178 | 907  | 794 |
| 2, 7 | —   | —   | 1                                       | 2  | 2   | 1   | Total   | 1518*   |     | 456                                     |     | 1701 |     |

\* Class (a) from X-XXX<sup>pd</sup> segregation. Other classes: (b) 80 XXY females (1 XXY female in control); (c) X<sup>p</sup>/X crossovers included in (a) and (b); (d) X<sup>d</sup>/X crossovers none; (e) 1 matroclinous female. From XXY mothers there were 26 matroclinous females and 28 patroclinous males.

Quota of chromatids used in determining percent of crossing over: 1518 from males of class (a), 80 represented by class (b); 1 represented by class (e).

TABLE 8

*Experiment 7. Offspring of Dp(1;f)/y<sup>2</sup> cv v B bb/y ec ct<sup>8</sup> s car ♀ by yXg ♂.*

| TYPE | NON-Dp<br>(♀ ♀) |      | CONTROL |     | TYPE    | NON-Dp<br>(♀ ♀) |    | CONTROL |   | TYPE       | NON-Dp<br>(♀ ♀) |      | CONTROL |     |
|------|-----------------|------|---------|-----|---------|-----------------|----|---------|---|------------|-----------------|------|---------|-----|
| 0    | 1054            | 1653 | 206     | 285 | 2, 7    | 3               | 7  | 6       | 1 | 1, 4, 6    | —               | —    | 1       | —   |
| 1    | 165             | 100  | 26      | 26  | 2, 8    | 1               | —  | 2       | 2 | 1, 7, 8    | 3               | —    | —       | —   |
| 2    | 229             | 170  | 32      | 39  | 3, 4    | 3               | 3  | 1       | 1 | 2, 3, 6    | 1               | —    | —       | —   |
| 3    | 314             | 223  | 46      | 31  | 3, 5    | 7               | 9  | 1       | 2 | 2, 4, 6    | —               | 1    | 1       | —   |
| 4    | 399             | 299  | 77      | 67  | 3, 6    | 11              | 15 | 5       | 6 | 2, 5, 8    | —               | —    | —       | 1   |
| 5    | 154             | 107  | 56      | 30  | 3, 7    | 2               | 4  | —       | 3 | 2, 6, 8    | —               | —    | 1       | —   |
| 6    | 186             | 113  | 68      | 58  | 3, 8    | —               | 1  | —       | 5 | 3, 4, 5    | —               | —    | —       | 2   |
| 7    | 17              | 16   | 30      | 19  | 4, 5    | 2               | 2  | 2       | 3 | 3, 4, 6    | —               | —    | 1       | —   |
| 8    | 20              | 10   | 23      | 9   | 4, 6    | 9               | 17 | 5       | 6 | 3, 4, 7    | 2               | 1    | —       | —   |
| 1, 2 | —               | 1    | —       | —   | 4, 7    | 2               | 1  | 8       | 5 | 3, 5, 7    | —               | —    | 1       | —   |
| 1, 3 | 2               | 1    | —       | —   | 4, 8    | —               | 2  | 2       | 6 | 3, 6, 8    | 1               | —    | —       | —   |
| 1, 4 | 12              | 12   | 6       | 3   | 5, 6    | —               | —  | 2       | — | 3, 7, 8    | —               | —    | 1       | —   |
| 1, 5 | 3               | 9    | —       | 2   | 5, 7    | —               | 2  | 1       | 1 | 4, 5, 6    | —               | 1    | —       | —   |
| 1, 6 | 7               | 25   | 3       | 13  | 5, 8    | —               | 1  | 1       | 7 | 4, 5, 8    | —               | —    | 2       | —   |
| 1, 7 | 3               | 2    | 6       | 3   | 6, 7    | —               | 1  | —       | — | 4, 6, 7    | —               | —    | —       | 2   |
| 1, 8 | —               | —    | 3       | 1   | 6, 8    | —               | 1  | 2       | 7 | 1, 4, 5, 8 | —               | 1    | —       | —   |
| 2, 4 | 4               | 15   | 2       | 8   | 7, 8    | —               | 2  | —       | — |            | 2627            | 2863 | 637     | 670 |
| 2, 5 | 4               | 11   | 4       | 5   | 1, 2, 4 | —               | —  | 1       | — | Total      | 5490            |      | 1307    |     |
| 2, 6 | 7               | 24   | 1       | 11  | 1, 3, 8 | —               | —  | 1       | — |            | (♂ ♂ = 6278)    |      | (1484)  |     |
|      |                 |      |         |     |         |                 |    |         |   | Total      | 11768*          |      |         |     |

\* Class (a) from X-XX<sup>nd</sup> segregation. Other classes: (b) 190 XXY females not equational, 105 (tested) of these not reciprocal X/X crossovers (no XXY females in control); (c) X<sup>p</sup>/X crossovers, from X-eggs, 5 apparent 7, 8 X/X crossovers, 3 of them also X/X crossovers at 1 (included in (a), (see table 2); X<sup>p</sup>/X crossovers from XX-eggs included in (b); (d) X<sup>d</sup>/X crossovers, 5 not-y non-Dp flies (2 females and 3 males) equally divided between complementary classes, and 1 Dp female carrying a y<sup>2</sup> (or y) fragment from X<sup>d</sup>/X crossing over (see table 2).

Quota of chromatids used in determining percent of crossing over: 5490 from females of class (a), 190 of class (b) and 2 of class (d).

TABLE 9

Offspring in lines of  $Dp(1;1)100$ . Experiment 1.  $Dp(1;1)$ ,  $y^2 cv v f/y ec ct^s car \varnothing$  by  $yXg \sigma$ . Experiment 10a.  $Dp(1;1)$ ,  $y^2 cv v f/y ec ct^s car/Y \varnothing$  by  $y^2 f B bb^1 \sigma$ .

| XXX <sup>pd</sup> |        |             | XX       |       | XXX <sup>pd</sup> Y |            | XXX <sup>pd</sup> |             |                | XX    |        | XXX <sup>pd</sup> Y |  |
|-------------------|--------|-------------|----------|-------|---------------------|------------|-------------------|-------------|----------------|-------|--------|---------------------|--|
| TYPE              | NON-Dp | Dp          | CONTROL  |       | NON-Dp              | TYPE       | NON-Dp            | Dp          | CONTROL        |       | NON-Dp |                     |  |
|                   | (♂ ♀)  | (♀ ♀)       | (♂ ♀)    | (♂ ♂) |                     |            | (♂ ♀)             | (♀ ♀)       | (♂ ♀)          | (♂ ♂) |        |                     |  |
| 0                 | 3279   | 1447        | 882      | 731   | 626                 | 4, 6       | 18                | 3           | 24             | 20    | 5      |                     |  |
| 1                 | 253    | (with 0)    | 76       | 71    | 59                  | 4, 7       | 3                 | 1           | 13             | 16    | —      |                     |  |
| 2                 | 414    | 152         | 110      | 109   | 74                  | 4, 8       | 3                 | (with 4, 7) | (with 4)       |       | 1      |                     |  |
| 3                 | 452    | 190         | 114      | 133   | 77                  | 5, 6       | —                 | —           | 2              | 1     | —      |                     |  |
| 4                 | 720    | 260         | 228      | 225   | 126                 | 5, 7       | —                 | 1           | 9              | 5     | 1      |                     |  |
| 5                 | 346    | 120         | 162      | 144   | 53                  | 5, 8       | 1                 | (with 5)    | (with 5)       |       | —      |                     |  |
| 6                 | 273    | 66          | 180      | 190   | 76                  | 6, 7       | —                 | —           | 1              | 3     | —      |                     |  |
| 7                 | 44     | 9           | 80       | 80    | 13                  | 6, 8       | 1                 | (with 6, 7) | (with 6)       |       | —      |                     |  |
| 8                 | 18     | (with 7)    | (with 0) |       | 3                   | 1, 2, 6    | 1                 | (with 2, 6) | —              | —     | —      |                     |  |
| 1 2               | —      | (with 2)    | —        | 1     | —                   | 1, 2, 7    | —                 | (with 2, 7) | 1              | —     | —      |                     |  |
| 1, 3              | 1      | (with 3)    | 2        | —     | 1                   | 1, 4, 5    | —                 | (with 4, 5) | 1              | —     | —      |                     |  |
| 1, 4              | 6      | (with 4)    | 12       | 13    | 2                   | 1, 4, 6    | 1                 | (with 4, 6) | 1              | —     | —      |                     |  |
| 1, 5              | 13     | (with 5)    | 10       | 7     | 4                   | 1, 5, 7    | 1                 | (with 5, 7) | 2              | —     | —      |                     |  |
| 1, 6              | 9      | (with 6)    | 11       | 20    | 5                   | 1, 6, 8    | 1                 | (with 6, 7) | (with 1, 6)    |       | —      |                     |  |
| 1, 7              | 1      | (with 7)    | 9        | 4     | —                   | 2, 3, 7    | —                 | —           | —              | 1     | —      |                     |  |
| 1, 8              | 1      | (with 7)    | (with 1) | —     | —                   | 2, 4, 5    | —                 | —           | 1              | —     | —      |                     |  |
| 2, 3              | —      | —           | 1        | —     | —                   | 2, 4, 6    | —                 | 1           | 1              | —     | —      |                     |  |
| 2, 4              | 8      | 2           | 16       | 15    | 3                   | 2, 4, 7    | 1                 | —           | —              | 4     | —      |                     |  |
| 2, 5              | 8      | 4           | 25       | 13    | 2                   | 2, 5, 7    | —                 | —           | —              | 2     | —      |                     |  |
| 2, 6              | 12     | 1           | 32       | 31    | 4                   | 3, 4, 6    | —                 | —           | 1              | —     | —      |                     |  |
| 2, 7              | 6      | 2           | 15       | 12    | 2                   | 3, 4, 7    | —                 | —           | 1              | 1     | —      |                     |  |
| 2, 8              | 4      | (with 2, 7) | (with 2) | —     | —                   | 3, 5, 6    | —                 | —           | —              | 1     | —      |                     |  |
| 3, 4              | 5      | 3           | 3        | 2     | 6                   | 3, 6, 7    | —                 | —           | —              | 1     | —      |                     |  |
| 3, 5              | 12     | 5           | 20       | 9     | 2                   | 4, 5, 7    | —                 | 1           | 2              | 1     | —      |                     |  |
| 3 6               | 6      | 1           | 16       | 14    | 4                   | 4, 6, 7    | —                 | —           | 1              | —     | —      |                     |  |
| 3 7               | 5      | 2           | 13       | 10    | —                   | 2, 5, 7, 8 | 1                 | —           | (with 2, 5, 7) |       | —      |                     |  |
| 3, 8              | 2      | (with 3, 7) | (with 3) | —     | —                   |            |                   |             | 2090           | 1898  |        |                     |  |
| 4, 5              | 6      | 2           | 12       | 8     | —                   | Total      | 5936*             | 2273        | 3988           |       | 1149†  |                     |  |

\* Exp. 1, class (a) from  $X-XX^{pd}$  segregation. Other classes: (b) 9  $XXX^{pd}Y$  females not equational (3  $XXY$  females in control); (c) one  $y^2 X^P/X$  crossover involving the free  $X$  (not tested), presumably an  $XX$  female, not equational (homozygosis for  $y^2$  was not detectable); (d)  $X^d/X$  crossovers none.

Quota of chromatids used in determining percents of crossing over: 5936 from males and females of class (a), 18 represented by class (b), 2 by class (c). Quota of control, 3988 males and females of class (a), 12 represented by class (b).

† Exp. 10a, class (a) from  $XY-XX^{pd}$  or  $X-XX^{pd}Y$  segregation. Other classes described in table 10.

Quota of chromatids used in determining percents of crossing over: 1149 from males of class (a) and (see table 10) 323 represented by class (b), 2 by diagonals of class (c) and 2 by crossovers with the free  $X$  of class (c).

TABLE 10

Experiment 9. Offspring of  $Dp(1;f)/y^2 cv v f bb^l/y ec c^R s car bb^l$  by  $y^2 B bb^l Y^{bb} \sigma^7$ .

Experiment 9d. Offspring of  $Dp(1;f)/y ec c^R s^2 car bb/y ec c^R s^2 car bb$  ♀ by  $y^2 B bb^l(Y^{bb}?) \sigma^7$ .

Experiment 1d. Offspring of  $Dp(1;1), y^2 cv v f/y w$  ♀ by  $y^2 cv v B \sigma^7$ .

Experiment 10b. Offspring of  $Dp(1;1), y^2 cv v f/y w/Y$  ♀ or  $Dp(1,1), y w cv v f/y^2/Y$  ♀ by  $y^2 cv v B \sigma^7$  and analysis of flies from two-X eggs in these experiments, in experiment 5a (including those from experiment 5, table 7, and additional matings) and in experiment 10a (table 9). For example, opposite (b), 2 = 0:1, 4 is the record of two tested females, of class (b), whose X's were one non-crossover and one double crossover in regions 1 and 4; [1] = 0:5 is the record of one not tested female, of class (b), whose X's were rated from the phenotype as one non-crossover and one crossover in region 5.

| PARENT ♀<br>EXP.                                       | OBSERVED ZYGOTES |      |      |                  | CROSSOVER CONSTITUTION OF X's (NUMBERS OF<br>FLIES NOT TESTED IN BRACKETS) |                              |                      |             |
|--|------------------|------|------|------------------|--|------------------------------|----------------------|-------------|
|  |                  |      |      |                  | XXXX <sup>pd</sup>   |                              | XXXX <sup>pd</sup> Y |             |
|  | 9                | 9d   | 1d   | 10b              | 9  | 5a                           | 10b                  | 10a         |
| Not X <sup>p</sup> /X crossovers                       |                  |      |      |                  |  |                              |                      |             |
| (a) X- or XY-eggs                                      |                  |      |      |                  |  |                              |                      |             |
| non-Dp ♀ ♀   | 15               | 1010 | 3140 | 4444             |  |                              |                      |             |
| non-Dp ♂ ♂   | 10476            | 1049 | 3058 | 4134             |  |                              |                      |             |
| (b) XX-eggs  | 594              | 26   |      |                  |  | 3084                         |                      | 1149        |
|  |                  |      |      |                  | [570] = 0:0  | [20] = 0:0                   |                      |             |
|  |                  |      |      |                  | 23 = 0:0   | 102 = 0:0                    |                      |             |
|  |                  |      |      |                  | [1] = 0:5  | 1 = 0:1                      |                      |             |
|  |                  |      |      |                  |  | 2 = 0:1, 4                   |                      |             |
|  |                  |      |      |                  |  | OR 1:4                       |                      |             |
|  |                  |      |      |                  |  | [1]? = 0:7                   |                      |             |
|  |                  |      |      |                  |  | 1 = 1:1                      |                      |             |
|  |                  |      |      |                  |  | 1 = 2:2                      |                      |             |
|  |                  |      |      |                  |  | 1 = 4:4                      |                      |             |
| XXXX <sup>pd</sup> -eggs                               |                  |      | 12   | 958              |  |                              | [80] = 0:0           | [206] = 0:0 |
|  |                  |      |      |                  |  |                              | 152 = 0:0            | 31 = 0:0    |
|  |                  |      |      |                  |  |                              | 2 = 0:1 OR 2         |             |
|  |                  |      |      |                  |  |                              | 3 = 0:3 OR 4         |             |
|  |                  |      |      |                  |  |                              | 1 = 1 OR 2:1         |             |
|  |                  |      |      |                  |  |                              | OR 2                 |             |
| O-eggs   |                  |      | 16   |                  |  |                              |                      |             |
| Y-eggs by  |                  |      |      |                  |  |                              |                      |             |
| X-sperm  |                  |      |      | 1046             |  |                              |                      | 323         |
| XX-sperm   |                  |      |      | 2 pat. ♀ ♀       |  |                              |                      |             |
| (c) X <sup>p</sup> /X crossovers                       |                  |      |      |                  |  | included in<br>(a) and (b)   |                      |             |
| X-eggs   | 4                | 1 ♂  |      |                  |  |                              |                      |             |
| XX-eggs  | 11               | 2    |      |                  | [11] = 0:0   |                              |                      |             |
| X <sup>p</sup> /X c.o. with at-<br>tached X (diagonal) |                  |      |      |                  |  |                              |                      |             |
| XX-eggs  |                  |      | 0    | 2                |  |                              | 1 = 0:0              | 1 = 0:4     |
|  |                  |      |      |                  |  |                              | 1 = 0:1 OR 2         |             |
| X <sup>p</sup> /X c.o. with free X                     |                  |      |      |                  |  |                              |                      |             |
| XX-eggs  |                  |      | 2*   | 2                |  |                              | [2] = 0:3 OR 4       | 1 = 0:0     |
|  |                  |      |      |                  |  |                              |                      | 1 = 0:2     |
| (d) X <sup>d</sup> /X crossovers                       | 1 ♂              | 0    | 0    | 0                |  | 0                            |                      |             |
| (e) XXX <sup>pd</sup> -eggs                            |                  | 1    |      |                  |  | 1                            |                      |             |
| (f) XX-eggs, equational                                | 1                |      | 1    | [1]?<br>(tested) | 1 = 0:5  | [1]? OR = 0:7<br>(see above) |                      |             |

\* Tested not X/X crossovers.

# GENETICS OF NATURAL POPULATIONS. II. GENIC VARIATION IN POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA* INHABITING ISOLATED MOUNTAIN RANGES

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Received April 19, 1938

## INTRODUCTION

POPULATIONS of *Drosophila pseudoobscura* inhabiting isolated mountain forests in the Death Valley region are highly variable with respect to gene arrangement as well as to gene content of the chromosomes. The first article of the present series (DOBZHANSKY and QUEAL 1938) dealt with the variability of the gene arrangement; the results of observations on the genic variation are to be reported here. The source of the material and the collecting methods have been described in the publication just quoted and need not be repeated, since the same samples of flies were used in both studies. Our experiments on the genic variation are confined to the third chromosome, although the species has five chromosome pairs. The reasons for this choice are purely technical: presence in the third chromosome of favorable gene markers associated with inversions facilitates the experimentation. Our results are essentially similar to those recently published by STURTEVANT (1937) and may be regarded as an extension of the latter. We are indebted to Messrs. EDWARD HELD and G. T. RUDKIN who have shared with us the routine work involved in conducting the experiments described in this article.

## DETECTION OF THE CONCEALED GENIC VARIABILITY

The samples of flies collected in the mountain forests of the Death Valley region appeared externally homogeneous. Of course, some individuals in these samples were larger and others smaller than the average, but such differences, whenever tested, were not inherited. They are due, presumably, to variations in the food supply of the larvae in the natural habitats. The apparent homogeneity of the samples does not prove however that the flies composing them are genetically identical. They may carry concealed recessive mutant genes. As pointed out by TSCHETWERIKOFF (1926), and by many others since then, special genetic methods are necessary for the detection of such concealed variability. The method which we used is as follows (fig. 1).

Each male collected outdoors (we shall call such males "wild males" and their chromosomes "wild chromosomes") was crossed to two or three

females homozygous for the third-chromosome recessives orange (*or*) and purple (*pr*), eye colors. If the number of wild males from a given locality was smaller than the sample desired, single sons of fertilized females caught in the same locality were also used. If any of the wild males is heterozygous for either *or* or *pr*, approximately one half of the offspring in the F<sub>1</sub> generation will manifest the effects of these mutant genes (table 4). For the detection of recessives other than *or* and *pr* the analysis must be carried two generations further.

Since every wild male has two third chromosomes, the F<sub>1</sub> generation consists of two indistinguishable (except where the wild male is heterozygous for *or* or *pr*) classes of individuals carrying one or the other of the wild third chromosomes. A single male from each F<sub>1</sub> culture was crossed to females having *or*, Blade (*Bl*), Scute (*Sc*), and *pr* in one third chromosome, and the "Cuernavaca" inversion in the other (fig. 1). Such females are phenotypically Blade (narrow wing) and Scute (some bristles absent); the inversion eliminates crossing over between the two chromosomes. In the next generation from the above cross three classes appear: wild type, *Bl Sc*, and *or Bl Sc pr*. The *Bl Sc* flies carry one wild third chromosome and one chromosome with the genes *or*, *Bl*, *Sc*, and *pr*; every *Bl Sc* fly in each culture carries the division products of the same wild chromosome present in the wild male.

Two or three virgin *Bl Sc* females were selected from each culture and crossed to four or five *Bl Sc* brothers. Three main classes of individuals are expected in the offspring (fig. 1). Individuals homozygous for the *or Bl Sc pr* chromosome are inviable because the gene *Bl* has a recessive lethal effect. Another class, *or Bl Sc pr*/wild, survives and shows *Bl* and *Sc* in the phenotype. The third class consists of flies homozygous for a wild third chromosome derived from the wild male ancestor; this class is most interesting to us. If the wild chromosome in question carries no mutant genes, this class is wild type in appearance; if the wild chromosome has a recessive mutant gene, or genes, producing visible external effects, the flies must be correspondingly modified; if, finally, the chromosome carries a recessive lethal, or lethals, no adult flies of this class can appear. In general, the non-*Bl* non-*Sc* flies must manifest the effects of any genetic factors that were present in the original wild chromosome.

The method just outlined for the detection of concealed genic variability is complicated by the fact that some crossing over may take place between the *or Bl Sc pr* and the wild chromosomes in the females. The possible crossover chromosomes are *or*, *Bl Sc pr*, *or Bl*, *Sc pr*, *or Bl Sc*, *pr*, *Bl Sc*, *or pr*, and others. It is easy to see that the eggs carrying these crossover chromosomes, fertilized by the *or Bl Sc pr* and wild type spermatozoa, will produce *Bl Sc*, wild type, *or Bl Sc*, *Bl Sc pr*, *Bl*, *Sc*, and *or Bl*

*Sc pr* individuals. Some of these individuals are homozygous for parts of the original wild chromosome, and hence may be normal, modified, or inviable, depending upon the genic contents of the respective parts of the wild chromosome. The difficulty is, fortunately, somewhat mitigated by the fact that the third chromosomes of the populations inhabiting the Death Valley region are variable in the gene arrangement. Three gene arrangements, known as "Standard," "Arrowhead," and "Chiricahua" are commonly found (DOBZHANSKY and STURTEVANT 1938, DOBZHANSKY and QUEAL 1938). Since the *or Bl Sc pr* chromosome has the Standard gene

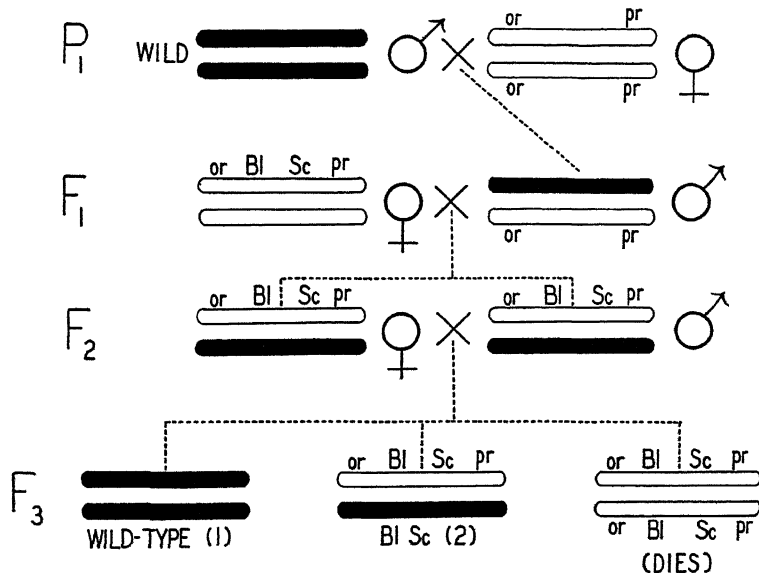


FIGURE 1.—A scheme of the experimental procedure used to obtain individuals homozygous for third chromosomes encountered in wild populations. The wild chromosomes are shown in black, those of laboratory strains in white.

arrangement, crossing over in the *or Bl Sc pr*/wild females can occur freely only if the wild chromosome also has the Standard arrangement. The frequency of crossing over in the Arrowhead/Standard and Chiricahua/Standard heterozygotes is so low that very few crossover individuals appear in the cultures. Furthermore, even if the wild chromosome has the Standard gene arrangement, the presence of lethals and other changes can be detected. Thus, if the wild chromosome of an *or Bl Sc pr*/wild female carries a lethal in the vicinity of the *Bl* locus, few or no non-Blade offspring are produced. With a lethal in the left end of the chromosome, for example in the vicinity of *or*, some Blade non-Scute but no Scute non-Blade individuals appear in the offspring, and if the lethal is located in the vicinity of *pr* the Blade non-Scute class is small or absent while the Scute non-Blade flies survive.



As a whole, the method is undeniably less rigorous when applied to Standard chromosomes than to those with other gene arrangements, but at least the detection of lethals is accurate irrespective of the gene sequence. In the following presentation the Standard wild chromosomes are treated separately from the Arrowhead and Chiricahua ones. One must also note that, since the detection of lethals is based on the absence of certain classes (chiefly the wild type class) in the offspring of the *or Bl Sc pr/wild* by *or Bl Sc pr/wild* crosses, the method does not discriminate between chromosomes having a single lethal and more than one lethal.

## LETHALS

A total of 849 wild third chromosomes (142 of them having the Standard gene arrangement) have been tested for the presence of lethals. These 849 chromosomes have come from ten samples from as many different localities (table 1). The average frequency of chromosomes carrying lethals proves to be  $11.9 \pm 0.75$  percent. This figure may be compared to that obtained by STURTEVANT (1937) for the third chromosomes of *D. pseudoobscura* coming from localities scattered widely over the distribution area of the species. STURTEVANT's figure is  $19.25 \pm 1.95$  percent, which

TABLE 1  
*Frequency of third chromosomes carrying lethals (in percent).*

| LOCALITY    | GENE<br>ARRANGEMENT      | FREQUENCY                          |                 | CHROMOSOMES<br>STUDIED |     |
|-------------|--------------------------|------------------------------------|-----------------|------------------------|-----|
| Lida        | Arr. and Ch.<br>Standard | $22.4 \pm 4.02$<br>0               | $20.0 \pm 3.64$ | 49<br>6                | 55  |
| Coso        | Arr. and Ch.<br>Standard | $16.0 \pm 2.27$<br>0               | $15.3 \pm 2.18$ | 119<br>5               | 124 |
| Cottonwood  | Arr. and Ch.<br>Standard | $16.4 \pm 3.20$<br>$9.4 \pm 3.48$  | $14.0 \pm 2.43$ | 61<br>32               | 93  |
| Grapevine   | Arr. and Ch.<br>Standard | $9.5 \pm 3.05$<br>$14.3 \pm 6.00$  | $10.7 \pm 2.79$ | 42<br>14               | 56  |
| Panamint    | Arr. and Ch.<br>Standard | $6.7 \pm 1.78$<br>$13.3 \pm 5.92$  | $7.6 \pm 1.76$  | 90<br>15               | 105 |
| Awavaz      | Arr. and Ch.<br>Standard | $5.6 \pm 3.66$<br>0                | $4.3 \pm 2.85$  | 18<br>5                | 23  |
| Kingston    | Arr. and Ch.<br>Standard | $15.2 \pm 2.98$<br>0               | $9.9 \pm 2.00$  | 66<br>35               | 101 |
| Charleston  | Arr. and Ch.<br>Standard | $12.5 \pm 2.38$<br>$6.7 \pm 4.36$  | $11.7 \pm 2.14$ | 88<br>15               | 103 |
| Sheep Range | Arr. and Ch.<br>Standard | $10.6 \pm 2.25$<br>0               | $10.0 \pm 2.14$ | 85<br>5                | 90  |
| Providence  | Arr. and Ch.<br>Standard | $11.2 \pm 2.25$<br>$20.0 \pm 8.53$ | $12.1 \pm 2.21$ | 89<br>10               | 99  |
| Total       | Arr. and Ch.<br>Standard | $12.9 \pm 0.85$<br>$7.0 \pm 1.44$  | $11.9 \pm 0.75$ | 707<br>142             | 849 |

is significantly higher than ours. The explanation of this difference is not clear, although one may note that most of the material studied by STURTEVANT came from regions that are more densely populated with *D. pseudoobscura* than the Death Valley region. In small populations the rate of elimination of lethals is expected to be greater than in large ones. DUBININ and his collaborators have analyzed 3924 wild second chromosomes of Caucasian populations of *D. melanogaster* and found that  $10.45 \pm 0.30$  percent of them carry lethals. This figure compares favorably with ours, but it must be kept in mind that the second chromosome of *D. melanogaster* composes a greater portion of the karyotype of that species than the third chromosome of *D. pseudoobscura*. On the assumption that the mutability per unit of the chromosome length is equal in both species, DUBININ's figure indicates a smaller accumulation of lethals in *D. melanogaster* than in *D. pseudoobscura*.

Chromosomes with the Standard gene arrangement have fewer lethals than Arrowhead and Chiricahua chromosomes ( $7.0 \pm 1.44$  percent and  $12.9 \pm 0.85$  percent, table 1). This difference is even more significant than it appears to be since some semilethals are probably included among lethals in the data for the Standard and not for other chromosomes (see below). Among the separate localities, some appear to have a higher concentration of lethals in the fly population than others (compare Lida and Coso with Panamint and Awavaz, table 1). No population, however, has been found to be free of lethals, and the figures for separate localities have probable errors so high that the significance of the differences is open to doubt.

#### SEMILETHALS

As stated above, inbreeding of the *or Bl Sc pr*/wild flies is expected to produce *Bl Sc* and wild type offspring in the ratio 2:1 (66.7 percent and 33.3 percent). Evidently, this expectation can be realized only if the viabilities of the individuals homozygous for the wild chromosomes and of the *or Bl Sc pr*/wild individuals are equal (fig. 1). This need not necessarily be true in practice. If the wild chromosome contains recessive or semi-dominant genes that cause a deterioration of the viability of the homozygote, the frequency of the wild type class will fall somewhere between 0 percent and 33.3 percent; presence of genes improving viability would, on the contrary, increase the frequency of the wild type class above 33.3 percent. The proportion of wild type flies appearing in the offspring of a given cross may serve, then, as a measure of the effects of the wild chromosome involved on the viability. Theoretically, the most serious limitation of this method is that the viability of each homozygote is compared not with that of a common standard type but with that of



the heterozygote for the *or Bl Sc pr* and the same wild chromosome. Since, however, the viability genes encountered in wild populations appear to be recessive this limitation is unimportant. Another limitation is due to the fact that the relative viabilities of two genetic types need not be the same under all possible environmental conditions; our experiments give us information on the viability merely under the conditions in which they are carried out. Finally, one could imagine that chromosomes other than the third (which is the only one which is being followed) contain genes that produce specific interactions with the third chromosome genes; this possibility is rather remote, and moreover the repetition of the test crosses in the following generation gives rather consistent results (see below).

The frequency of each class of flies appearing in the offspring of the *or Bl Sc pr*/wild by *or Bl Sc pr*/wild cultures was determined. Five counts were made in every culture, the first being made two or three days after the adult flies began to emerge, and the following ones at three day intervals thereafter. The records for the 849 cultures are far too bulky to be published, but one may note that the total numbers of the flies per culture fall mostly between 200 and 300, and only very rarely below 100. The number of the non-Blade (mostly wild type) flies was expressed in percent of the total number of flies in the culture. As stated above, the frequency of wild type flies may serve as a measure of the viability of the type homozygous for a given wild third chromosome. A summary of the data is presented in the form of variation series in table 2. This table shows, consequently, the number of cultures in which a given percentage (from 0 percent to 50 percent) of wild type flies was observed.<sup>1</sup>

The frequencies of the wild type (or non-Blade) flies in different cultures range from 0 to almost 50 percent. The distribution curve (graphically represented for the non-Standard chromosomes in fig. 2) has an interesting shape. The zero class, including the lethals, has a frequency of 13 percent (see above). Classes above zero but below 14 percent are represented by

<sup>1</sup> The following convention was adopted in computing the values to be entered in table 2. If the non-Blade flies composed more than 15 percent of the total, their frequency was entered directly. If they amounted to less than 15 percent, indicating that the culture in question carries a lethal or a semilethal, their origin was examined further. With a lethal lying in the chromosome far from the locus of *Bl*, some non-*Bl* flies appear as a result of crossing over; the non-*Bl* flies, however, may also represent the surviving homozygotes for a third chromosome carrying a semilethal. In the former case the wild types are vigorous and normal in appearance, in the latter they usually are weak and abnormal. The non-*Bl* crossovers were disregarded and the surviving homozygotes counted. In case of doubt further tests were made by breeding the presumed crossovers and examining other crossovers appearing in the same culture. In cultures where the wild chromosome has a gene arrangement other than Standard such tests yield conclusive results, but where Standard is involved the surviving semilethal homozygotes are easily confused with the numerous crossovers. This consideration probably explains the fact that no semilethals in Standard chromosomes are recorded in table 2. If they were present in the material, they are included among the complete lethals (class 0).

26 cultures, or 3.7 percent of the total. These may be called the semilethals. Classes from 14 percent to 18 percent are not represented, and those from 18 percent to 50 percent form an apparently regular probability distribution with a mode in the 32 percent to 34 percent class, that is, around the theoretically expected value of 33.3 percent.

The distinction between the lethals and the semilethals as classes is not a sharp one. Chromosomes that in one generation produce a few surviving homozygotes, and are therefore classed as semilethals, may in another test produce none, or vice versa. This is a common experience in *Drosophila* genetics, particularly in mutation studies. Among the 849

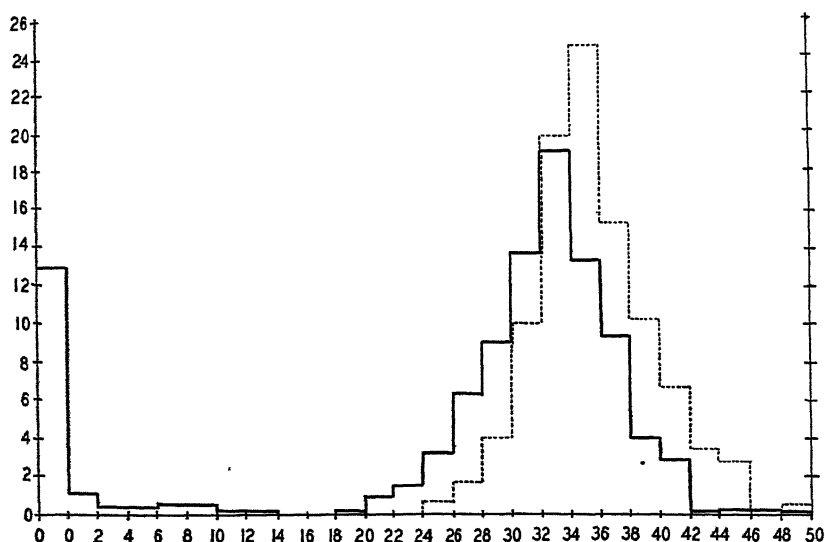


FIGURE 2.—The percent of wild type flies obtained in cultures in which these flies are homozygous (solid line) and heterozygous (dotted line) for individual third chromosomes encountered in wild populations. Horizontal axis—percents of wild type flies; vertical axis—percents of cultures in which a given frequency of wild type flies has been observed. Further explanation in text.

chromosomes tested, 127, or 15 percent, carry lethals or semilethals. Therefore, only 72.25 percent of flies in natural populations may be expected to be free of lethals, 25.50 percent to carry a lethal in one third chromosome, and 2.25 percent a lethal in both third chromosomes. The last class survives provided the two lethals are not alleles, which is frequently the case.

#### MINOR VARIATIONS IN THE VIABILITY

The part of the curve extending from 18 percent to 50 percent resembles a probability distribution (table 2, fig. 2). The variation within these limits may be due either to chance deviations from the expected value of 33.3 percent, or to presence of genetic factors causing minor deviations

from the "normal" viability, or to both. It is important to discriminate among these possibilities. If the frequency of the wild type class is determined by counting 200 flies, the probable error of the expected frequency, 33.3 percent is  $\pm 2.25$  percent. Since the average number of flies per culture in our experiments was greater than 200 (see above), the observed variance of the curve is too large to be accounted for by sampling errors only. This fact alone makes it probable that genetic modifiers of the viability are involved, the more so since values between 18 percent and 26 percent and between 40 percent and 50 percent are not uncommonly obtained in cultures producing more than 200 flies. Further experiments were made to test the validity of this inference.

Up to this point we have discussed the results of the intercrossoes of the *or Bl Sc pr*/wild individuals obtained in the offspring of the *or pr*/wild by *or Bl Sc pr*/Cuernavaca crosses (fig. 1). It may be noted that, disregarding the occurrence of crossing over, the *Bl Sc* individuals appearing in the last generation represented in figure 1 are identical in genetic constitution with their *Bl Sc* parents (that is, the constitution is *or Bl Sc pr*/wild). If we intercross them, the offspring is expected to consist again of 33.3 percent wild type and 66.7 percent of *Bl Sc* individuals. If, however, the wild chromosome involved in a given experimental culture carries genes decreasing or increasing the viability of the homozygotes compared to the average, the same deviations from the expected values may occur in both generations in each line. In other words, the presence of genes modifying the viability will manifest itself in a positive correlation between the frequencies of the wild type individuals obtained in the succeeding generations. Contrariwise, if the probability distribution shown in figure 2 is due to environmental variations or to sampling errors, there will be no correlation between these frequencies.

The amount of labor involved in raising one more generation for every one of the 849 chromosome-lines studied seemed to be prohibitive. As a compromise, the samples from Coso, Panamint, and Charleston mountains were selected and the experiment made with them. Moreover, since the Standard wild chromosomes are likely to be broken up by crossing over in the first generation, they were disregarded, and only the Arrowhead and Chiricahua chromosomes were used. The distinction between the Standard chromosomes and others is, of course, clear from the ratios observed in the first generation, since the former produce many more crossovers than the latter. The results may be represented best in the form of correlation tables in which the frequencies of the wild type (non-*Bl*) individuals obtained in the first generation (that is, the last generation shown in fig. 1) are indicated on one axis and the frequencies of the same class of individuals in the second generation on the other axis.

Figure 3 is such a correlation table for the combined data for the samples from the three localities mentioned above.

The two upper and lower left quadrants into which the correlation table shown in figure 3 is divided are indicative of the variable effects of some of the lethals and semilethals. Thus, one may see that one of the strains which in the first generation produced no wild type individuals, and hence was classed as containing a lethal, has produced between ten

| 1st Generation<br>2nd Generation | 0  | 0-2 | 2-4 | 4-6 | 6-8 | 8-10 | 10-12 | 12-14 | 14-16 | 16-18 | 18-20 | 20-22 | 22-24 | 24-26 | 26-28 | 28-30 | 30-32 | 32-34 | 34-36 | 36-38 | 38-40 | 40-42 | 42-44 | 44-46 | 46-48 | 48-50 |     |
|----------------------------------|----|-----|-----|-----|-----|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| 0                                | 11 | 1   |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 14  |
| 0-2                              |    | 1   |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1   |
| 2-4                              |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1   |
| 4-6                              |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1   |
| 6-8                              |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1   |
| 8-10                             |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1   |
| 10-12                            | 1  |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 3   |
| 12-14                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1   |
| 14-16                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1   |
| 16-18                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1   |
| 18-20                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 3   |
| 20-22                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 9   |
| 22-24                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 10  |
| 24-26                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 28  |
| 26-28                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 34  |
| 28-30                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 34  |
| 30-32                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 30  |
| 32-34                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 12  |
| 34-36                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 9   |
| 36-38                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 7   |
| 38-40                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 6   |
| 40-42                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 5   |
| 42-44                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 211 |
| 44-46                            |    |     |     |     |     |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |     |
|                                  | 12 | 2   | -   | -   | -   | -    | -     | -     | -     | -     | -     | 8     | 6     | 18    | 17    | 28    | 47    | 31    | 22    | 5     | 5     | 1     | 1     | 1     | 1     | 1     |     |

FIGURE 3.—Percent of wild type flies produced in a given line in two subsequent generations. Further explanation in text.

and twelve percent of wild types in the second generation, passing over into the semilethal class. Conversely, three of the strains behaved as lethal bearing in the second but not in the first generation.

Most important for our purposes is, however, the lower right quadrant of figure 3, containing the records for the strains that have produced more than 18 percent of wild type individuals in both generations. This quadrant may be treated as an independent correlation table, and computing the numerical value of the correlation coefficient according to the Bravais formula we obtain  $r = +0.379 \pm 0.062$ , that is, a distinct positive correlation. The table shown in figure 3 contains, as stated above, the

combined data for the Coso, Panamint and Charleston samples. Taking the samples separately, the correlation coefficients are  $r = +0.220 \pm 0.11$  for Coso,  $+0.517 \pm 0.094$  for Panamint and  $+0.451 \pm 0.101$  for Charleston. Except in the Coso sample, the positive correlation is undoubtedly significant. It follows that the variations of the frequencies of the wild type individuals in our experiments are in part due to genetic causes, and consequently, in natural populations, the third chromosomes may contain not only lethals and semilethals but also genes producing relatively minor viability effects.

The question that immediately presents itself is how frequent are chromosomes carrying such minor viability changes? In attempting to answer this question a difficulty is encountered. A "change" logically presupposes a standard of comparison; in treating of viability changes an implied assumption is made that the viability of the experimental strain is compared with that of a strain endowed with a certain "normal" viability. But what is the normal viability of *Drosophila pseudoobscura*? That of the Blade Scute flies is not necessarily normal, since these flies carry the two dominant mutant genes just named, and the mutants frequently reduce the viability of their carriers. Evidently, the normal viability must be looked for elsewhere.

#### THE "NORMAL" VIABILITY

Our experiments were designed to produce flies homozygous for individual third chromosomes present in wild populations. Herein lies an important difference between the wild type flies obtained in the experimental cultures and the wild flies living outdoors. Certainly, individuals homozygous for the descendants of a given chromosome may occasionally be produced in nature as well as in experiments, but, except in very small and highly inbred populations, such individuals are probably not very common in natural habitats. Provided many chromosome types differing in gene content are present in a population, and provided interbreeding of members of different families takes place, most wild flies ought to be heterozygous for unlike chromosomes of each kind. The viability of such individuals may, then, serve as a fair standard of comparison. It is easy to produce such individuals experimentally.

Instead of intercrossing the *or Bl Sc pr*/wild females and males coming from the same culture (fig. 1), one may make similar crosses using females from one culture and males from another. Since in every culture the descendance of one particular third chromosome is perpetuated, the wild type individuals obtained in the offspring of such a cross will necessarily have two third chromosomes of different origin. To make the experimental conditions approach as nearly as possible the natural ones, the crosses



were made between different lines derived from the same locality. Thus, if our fly samples had come from inbred populations, the chance of obtaining homozygotes instead of heterozygotes would be equal to the chance of such a happening in nature. Moreover, in order to test the recessiveness of the lethals the experiment was subdivided into two parts: in one the flies were obtained free of lethals, and in the other flies were made heterozygous for two lethal-bearing chromosomes.

In the offspring of both types of crosses a segregation in the ratio 66.7 percent *Bl Sc*:33.3 percent wild type is expected, provided, of course, that the viability of the wild type is equal to that of the *Bl Sc* flies. (In order to avoid complications due to crossing over, chromosomes with the Standard gene arrangement were not used in these experiments.) The results are summarized in table 3. The proportion of wild types varied in

TABLE 3

*Viability of types heterozygous for two different third chromosomes extracted from wild populations.*

|  | 24-26 | 26-28 | 28-30 | 30-32 | 32-34 | 34-36 | 36-38 | 38-40 | 40-42 | 42-44 | 44-46 | 46-48 | 48-50 | TOTAL |
|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Free of lethals                        | 1     | 3     | 2     | 8     | 18    | 20    | 15    | 10    | 9     | 5     | 5     | —     | 1     | 97    |
| Heterozygous for two different lethals | —     | —     | 5     | 10    | 17    | 24    | 12    | 8     | 3     | 1     | —     | —     | —     | 80    |
| Total                                  | 1     | 3     | 7     | 18    | 35    | 44    | 27    | 18    | 12    | 6     | 5     | —     | 1     | 177   |

different cultures from 25.8 percent to 49.2 percent, the mode falling in the 34 percent to 36 percent class. The distribution curve is represented in figure 2.

The curve for the heterozygotes, although broadly overlapping that for the homozygotes, is displaced toward the right (fig. 2). Hence, the average viability of the types homozygous for third chromosomes found in wild populations is lower than that of the presumed heterozygotes. The mean frequencies of the wild types for heterozygotes and homozygotes are  $35.52 \pm 0.20$  percent and  $32.58 \pm 0.12$  percent respectively. In computing these figures only Arrowhead and Chiricahua chromosomes were used, and the parts of the distribution involving the lethals and semilethals (table 2, fig. 2) were disregarded.

An analysis of these figures leads to interesting conclusions. The frequency of the wild type class in the cultures where this class is, by definition, endowed with "normal" viability is  $35.52 \pm 0.20$  percent, that is, significantly higher than the theoretically expected frequency of 33.33 percent. The origin of this discrepancy is clear enough. In these cultures the "normal" viability of the wild-types is measured against that of the *Bl Sc* class,

hence the discrepancy is due to adverse effects on the viability of the two dominant genes, *Bl* and *Sc*. On the other hand, in the cultures in which the wild type flies are homozygous for the wild third chromosomes the average frequency of these flies is  $32.58 \pm 0.12$  percent, that is, just significantly below 33.33 percent. It follows, then, that the average reduction of viability produced by homozygosis for wild third chromosomes is slightly greater than that caused by the simultaneous presence of the two mutant genes, *Bl* and *Sc*. Although these mutants are considered, as mutants go, fairly "good," both of them produce, in heterozygous condition, rather drastic modifications of the appearance of the fly, and would hardly be expected to persist in the natural state.

The conclusion follows that, aside from the wild third chromosomes carrying lethals and semilethals, a considerable proportion of these chromosomes contains recessive genetic factors that decrease the viability to a greater or lesser extent, the average being comparable to the combined effects of *Bl* and *Sc*. How great is the proportion of such chromosomes in the populations studied can be answered only as a rough approximation. The difficulty is that the chromosomes carrying deleterious genes do not form a clearly delimited group but overlap the "normal viability" distribution. To arrive at some estimate, we may use the correlation table shown in figure 3, which contains data for 188 chromosomes which in two generations have given more than 18 percent of wild type flies in the cultures, and consequently are free from lethals and semilethals. Among these, 82 chromosomes have given in both generations less than 34 percent of wild type flies, which may be taken as evidence of the reduction of the viability of the homozygotes. Although some of the lines might have twice produced low ratios due to chance alone, there must be others with a real reduction of the viability that in one or the other generation have also by chance given a normal ratio. Since the total number of the chromosomes is 211 (fig. 3), the 82 form 38.9 percent of the total. This is the approximation sought for.

An examination of table 2 and of figures 2 and 3 shows furthermore that in some cultures the frequency of wild types is above 40 percent of the total. This suggests that some wild chromosomes are not only free of deleterious recessives, but even carry genetic factors improving the viability of the fly above the average for either homozygotes or heterozygotes, at least under the environmental conditions prevalent in our experiments. In figure 3 we find that 4 cultures among 211 gave more than 40 percent of wild types in two generations, which suggests that about 1.9 percent of wild third chromosomes carry favorable viability genes. We may emphasize again that we have no illusions as to the accuracy of these estimates, although we are inclined to regard them as minimum ones.

Our experiments were planned to detect concealed recessive viability factors present in wild populations. Since recessiveness and dominance are relative one may inquire whether the factors detected are completely or only partly recessive. This question is least difficult to decide where lethals are involved. As mentioned above, the experiment with the heterozygotes for two different wild chromosomes was subdivided in two parts, in one of which the intercrossed *Bl Sc* flies were free of lethals in the third chromosome, and in the other both parents carried non-allelic lethals (table 3). Consequently, the wild types obtained in the first series carry no lethals, and the *Bl Sc* flies carry only *Bl*. In the second series the wild types are heterozygous for two lethals, and the *Bl Sc* flies for only one (disregarding *Bl*). If the lethals are incompletely recessive, the proportion of wild types in the cultures of the first series should be higher than in the cultures of the second series. Computing the average frequency of wild types from the data presented in table 3, we obtain the figures  $36.18 \pm 0.31$  percent and  $34.72 \pm 0.23$  percent for the first and second series respectively. The difference is  $1.46 \pm 0.39$  percent, which is statistically significant. Taken at their face value, these data indicate, then, an incomplete recessivity of at least some lethals. This question needs further study; the intercrosses involving lethals were not done simultaneously with those free of lethals, hence the results obtained may be due simply to environmental variations over a period of two or three months.

#### MODIFIERS OF THE DEVELOPMENT RATE

As stated above (p. 469), five counts, spaced at three day intervals, were made in every culture containing the *or Bl Sc pr*/wild by *or Bl Sc pr*/wild crosses. The cultures were kept in an incubator at  $24.5^{\circ}\text{C}$ , the environment having been made as homogeneous as possible. In examining the records thus obtained we have made the following observation. In some cultures the proportion of the wild type flies in the first count was very much below the expected frequency of 33.3 percent, and sometimes no wild types at all were obtained; but in the later counts in the same cultures the frequency of wild types rose much above 33.3 percent. Other cultures produced an excess of wild types in the first count, followed by a deficiency in the later ones. It is especially common for the cultures containing semilethal third chromosomes to produce some wild type flies in late counts only.

This observation suggested that some wild third chromosomes carry genetic factors which, in homozygous condition, produce either a slowing down or a speeding up of the development of the fly. Indeed, if the development rate of the homozygotes is low, a deficiency of wild types must be observed in early and an excess in late counts; if the wild type flies develop

faster than their *Bl Sc* sibs, an excess of the former must be observed in early counts and a deficiency in the late ones.

To test this hypothesis the following procedure was applied. A deviation from the expected ratio of wild types in any count may be due either to chance or to a modification of the development rate; in the former case the observed deviation is just as likely to be followed in a later count by a deviation of the same or of the opposite sign. But if modifiers of the development rate are present, deficiencies of wild types in early counts must be followed by excesses in later ones, or vice versa. In other words, the presence of modifiers will manifest itself in a negative correlation between the proportions of the wild types observed in different counts. The problem becomes more involved due to the presence of viability modifiers in our material: the ratios of the wild types to *Bl Sc* observed in different counts in individual cultures are bound to show a positive correlation, which may conceal the negative one produced by the modifiers of the development rate. To eliminate this source of error, a correction must be introduced in the data.

The observed percentages of the wild types in the first and in the third counts were computed for cultures carrying no lethals or semilethals giving less than 5 percent of wild types in total counts. The expected ratio of wild types, that is, 33.3 percent, was divided by the ratio of wild types observed in the total counts in a given culture; the figure thus obtained is the correction factor for the culture in question. The observed frequencies of the wild types in the first and the third counts were then multiplied by the correction factor, and the products entered into the correlation tables. The correlation coefficients for the samples studied are as follows:

|          |                                   |             |                        |
|----------|-----------------------------------|-------------|------------------------|
| Panamint | $r = -0.255 \pm 0.103$            | Sheep Range | $r = -0.385 \pm 0.100$ |
| Kingston | $r = -0.467 \pm 0.104$            | Providence  | $r = -0.318 \pm 0.102$ |
|          | Charleston $r = -0.420 \pm 0.095$ |             |                        |

All the correlation coefficients are negative. It may be noted that the correlation is determined between ratios observed in the first and the third counts only, the cultures that make the correlation negative are those in which the deficiency or excess of wild types observed in the first count has *already* been replaced by the opposite condition in the third count. Yet, some chromosomes slow down the development to such an extent that the wild types appear in greatest numbers only toward the end of the life of the culture, in the fourth or fifth counts. Such cultures weaken the negative correlation observed instead of strengthening it.

An independent evidence for the existence of modifiers of the development rate was secured by a different method. The samples from Panamint,

Charleston, and Coso mountains were tested in two generations (see above). It is evident that if genetic modifiers of the development rate occur, similar deviations from the expected ratios of wild types are to be expected in corresponding counts in both generations. Contrariwise, if the deviations are due to random sampling, the sign of the deviation in one generation will bear no relation to that in the next one. In other words, genetic modifiers must produce a positive correlation between the frequencies of the wild types in the corresponding counts in the two generations. These frequencies were computed for the corrected first and the third counts in the Panamint and Charleston samples. The correlation coefficients are as follows:

|            | First count           | Third count           |
|------------|-----------------------|-----------------------|
| Panamint   | $r = +0.31 \pm 0.122$ | $r = +0.05 \pm 0.133$ |
| Charleston | $r = +0.63 \pm 0.085$ | $r = +0.42 \pm 0.113$ |

Except for the third counts in the Panamint sample, a positive correlation is undoubtedly present. The existence of recessive modifiers of the development rate is established. What proportion of the chromosomes in wild populations carry such modifiers cannot be estimated even approximately from the data now available; one of us is planning to undertake special experiments to elucidate this question. For the time being one may note that cultures suggesting presence of these modifiers have been encountered in the populations of flies from every one of the ten localities studied. Flies homozygous for wild third chromosomes develop in some cultures slower and in others faster than their *Bl Sc* sibs. This shows that modifiers slowing down the development, as well as those speeding it up, are encountered in wild populations, although in looking over the data an impression is gained that retardations are decidedly more common than accelerations. In general, the chromosomes containing semilethals or genes reducing the viability mostly cause also retardations of the development. This rule is, however, by no means free of exceptions. Some semilethal homozygotes hatch early, and some slowly hatching types finally emerge in large numbers. The fast hatching types usually have a good viability, but at least one exception has been found.

#### MUTATIONS PRODUCING VISIBLE EXTERNAL EFFECTS

The genetic variability discussed so far concerns so-called physiological characters, vitality and development rate. Wild populations also carry some recessive mutants producing alterations of the external appearance of the fly. Of course, there is no sharp dividing line between "physiological" and "morphological" variations. Most of the flies homozygous for semilethals are small or otherwise abnormal in appearance, so that these semilethals might just as well be classed as "visibles."

In our experiments wild males and single sons of wild females were crossed to females homozygous for the third chromosome recessive eye color genes orange and purple (fig. 1). In some cultures one half of the offspring showed one or the other of these mutant characters, proving that the father was heterozygous for it. Purple has been found in five chromosomes from three localities and orange in a single chromosome (table 4).<sup>2</sup>

TABLE 4  
*Occurrence of the mutants purple (pr) and orange (or) in wild populations.*

| LOCALITY   | CHROMO-<br>SOMES<br>TESTED | <i>pr</i> | <i>or</i> | LOCALITY    | CHROMO<br>SOMES<br>TESTED | <i>pr</i> | <i>or</i> |
|------------|----------------------------|-----------|-----------|-------------|---------------------------|-----------|-----------|
| Lida       | 132                        | —         | —         | Awavaz      | 52                        | 1         | —         |
| Coso       | 298                        | —         | —         | Kingston    | 246                       | —         | 1         |
| Cottonwood | 244                        | —         | —         | Charleston  | 238                       | 2         | —         |
| Grapevine  | 144                        | —         | —         | Sheep Range | 242                       | —         | —         |
| Panamint   | 272                        | 2         | —         | Providence  | 274                       | —         | —         |

In the critical generation of our series of crosses (the last generation shown in fig. 1) the non-*Bl Sc* flies in some cultures manifested various peculiarities, suggesting the presence in the wild third chromosomes of mutant genes with visible external effects. All suspected mutants were tested for inheritance. Nevertheless, we do not feel that our data on the frequency of the visibles are accurate. Aside from the fact that some mutants may have been overlooked (we do not claim to be good mutation finders), some cultures apparently contained genetic changes that manifest themselves only in a part of the flies carrying them. The progeny tests were not extensive enough, and some cultures were discarded if they failed to show the mutant character in one generation; a part of them might have contained real mutants. The number of chromosomes carrying undoubted mutants is shown in table 5. The semilethals that produce in

<sup>2</sup> Orange, purple, the second chromosome recessive cinnabar, and certain other mutants appear to be rather common in wild populations of *D. pseudoobscura* inhabiting the western part of the species area. In the experiments of Dr. A. H. STURTEVANT and others in this laboratory, these mutants have appeared in the stock cultures of the following lines, mostly a few generations after these lines were established from wild ancestors. Orange, Race A: Lake Okanagan-8, 9 (British Columbia), Dunsmuir-4, Lassen-16, Banner-10 (California); Race B: Olympic-5, The Dalles-7 (Washington), Klamath-5, Sequoia Park-16, 17 (California). Purple, Race A: Chelan-3, 7, 12 (Washington), Cinnabar; Race A: Dollar Lake-3 (California); Race B: Humboldt-5, Lassen-12, 17, 18, 20 (California). It has to be kept in mind that fewer cultures were examined from all these localities combined than from the Death Valley region alone, and that no special crosses for the detection of mutants were made. In more extensive material from the region east of the Sierra Nevada—Cascades Mountains orange was found only once (Santa Catalina-6, Arizona).

homozygous condition some external abnormalities are not included in this table.

TABLE 5  
*Number of wild third chromosomes carrying visibles.*

| LOCALITY   | CHROMO-<br>SOMES<br>TESTED | NUMBER<br>OF<br>MUTANTS | LOCALITY    | CHROMO-<br>SOMES<br>TESTED | NUMBER<br>OF<br>MUTANTS |
|------------|----------------------------|-------------------------|-------------|----------------------------|-------------------------|
| Lida       | 55                         | 0                       | Awavaz      | 23                         | 1                       |
| Coso       | 124                        | 3                       | Kingston    | 101                        | 7                       |
| Cottonwood | 93                         | 3                       | Charleston  | 103                        | 2                       |
| Grapevine  | 56                         | 1                       | Sheep Range | 90                         | 2                       |
| Panamint   | 105                        | 5                       | Providence  | 99                         | 7                       |

Among the 849 chromosomes tested, 30 chromosomes, or 3.5 percent, contained visibles. The salient characteristics of some of the mutants are described below.

*A weak allelomorph of purple.* Found in five chromosomes from Kingston and in one chromosome from Awavaz mountains. Flies homozygous for this gene are similar to the wild type, the eye color being only very slightly purplish. The heterozygotes carrying weak purple in one chromosome and purple and orange in the other have an eye color intermediate between that found in the homozygous purple and in wild type; in the males of this genetic constitution the testicular envelope is yellowish orange, instead of bright red as in wild type or transparent as in homozygous purple. Flies homozygous for orange and heterozygous for purple and for weak purple have bright yellow eyes, instead of the bright red ones which appear in the absence of weak purple. Classification of weak purple is reliable only in flies homozygous for orange. The former may be described as a weak wild type allelomorph which is only incompletely dominant over the mutant purple. The occurrence of such an allelomorph in wild populations is of interest in connection with the general problem of the origin of dominance. Furthermore, it should be noted that 35 wild third chromosomes with the standard, and 66 chromosomes with other gene arrangements were analyzed in the sample from the Kingston Range, and that weak purple has been found five times in the former and not at all in the latter. Therefore, about one-seventh of the Standard chromosomes in this locality carry weak purple. In all other samples studied by us weak purple has been encountered only once, namely in a Standard chromosome from Awavaz. It is virtually certain that the five Kingston chromosomes carrying weak purple are of a common origin, and hence this mutant is for some reason spreading in the population inhabiting this locality. Whether the single chromosome found in the Awavaz population

is derived from the same source as the Kingston findings is an open question.

*Short wings.* The wing is about one fifth shorter than normal, the tip is somewhat rounded. Found four times in the sample from Providence mountains, and once in Coso. Three of the Providence mutants proved to be allelic to each other but not to the fourth. The Coso mutant has not been tested. Although the visible effects of these mutants are so slight that they may be easily overlooked, their triple occurrence in the Providence sample is suggestive.

*Dwarfish.* Fly smaller than normal in size. This type of mutant is common in wild populations, but body size is so much influenced by environmental conditions that the classification is not accurate. Found once in each Cottonwood, Panamint, Sheep Range, and Providence, and twice on Kingston Range. Allelism not tested. Some of the semilethals appear also as dwarfs, but they are not included in the above count.

*Short or thin bristles.* This is another common mutant type, which is even more easily overlooked than dwarfishness, since small bristles are frequently produced as a modification. It was found once in Coso and Grapevine, and twice in Panamint.

*Polished body.* The body surface, except for the intersegmental membranes, is shiny, with a slight metallic lustre. Wings spoon-shaped, rather opaque. All bristles and the microchaetae are present, so that the alteration of the appearance of the body surface must be due to a change in the microstructure of the chitin. Viability is normal. This excellent mutant has been found in a single chromosome in Charleston.

*Plexus venation.* A rather extreme allelomorph of plexus first discovered by STURTEVANT (STURTEVANT and TAN 1937) in a wild strain from British Columbia. Ours was found in one chromosome from Providence.

*Vestigial-like.* Wings are notched or reduced in various degrees. In some individuals wings are normal in length but have nicks or notches on the tip or on the inner margin; in others the notching is so extensive that the wing resembles antlered or strap; in still others it is vestigial. The eye shape and size vary from normal to very small and knob-like. The scutellar bristles are normal. The phenotype of this mutant strikingly resembles that of the sex-linked recessive recently found by STURTEVANT (undescribed), albeit ours is located in the third chromosome. The two are not allelomorphs. This is one of the best examples of mimic mutants on record. The variations in the wing shape are observed even in flies hatching in the same culture; they parallel most of the known allelomorphs of the gene vestigial in *D. melanogaster*. This mutant was observed in a single chromosome from Providence.

*Curved-like wing.* Resembles the mutant curved described by STURTEVANT



and TAN (1937). Homozygotes are sterile, but whether the sterility is due to an accessory effect of the curved locus or to a separate gene is unknown. The allelism of curved and curved-like has not been tested. The mutant was found in one chromosome from Charleston.

*Curly-like wing.* Wings are curled upwards and held divergent from the body. Some bristles on the head, thorax, and scutellum are absent, the bristle pattern is irregular, asymmetry is frequent. This was found in one chromosome from Cottonwood.

*Abnormal abdomen.* The chitin of the abdominal sclerites is thin and soft. In extreme specimens the whole abdomen appears covered by intersegmental membrane, and is therefore rounded and shriveled. Homozygotes are sterile. This was found in one chromosome from Panamint.

*Chlorotic body color.* Color yellowish-brown, turning a translucent greyish-yellow where chitin is thin. Wings yellowish, somewhat opaque. Homozygotes are sterile. The mutation was found in one chromosome from Panamint.

*Wavy wings.* The wing surface is uneven, the tips of the wings frequently turned upwards (like jaunty of *D. melanogaster*) or downwards (like arc). Probably non-allelic mutants of this type were found in Coso, Cottonwood, and Sheep Range, one chromosome in each. Here belong also two somewhat doubtful mutants from Providence and Panamint.

The above data, though not as extensive as one may wish, are sufficient to show that wild populations carry a variety of recessive genes with visible external effects, ranging from relatively slight "minor" to sharp "major" changes.

#### CONCLUSIONS

The external uniformity of wild populations of *D. pseudoobscura* is spurious, since it conceals a wealth of recessive variations carried in heterozygous conditions. In the populations from the Death Valley region, 11.9 percent of wild third chromosomes carry lethals, 3.1 percent semilethals, about 39 percent deleterious genetic factors having too slight effects to be classed as semilethals, and about 2 percent carry factors increasing the viability above the average. Furthermore, no less than 3.5 percent of the wild third chromosomes contain recessive mutant genes with visible external effects, and a fraction which we cannot yet estimate quantitatively contain genes modifying the development rate. It is very probable that further studies would detect also genetic variations affecting other characteristics, such as fertility, length of life, etc. The ten samples from different localities all behaved essentially alike, giving us reason to believe that the conditions found in these samples are universal, at least for wild populations of *D. pseudoobscura*.

Certain biologists have put forward assertions that genetic variations of the mutation type are laboratory products, and do not occur at all, or at least are very rare and "abnormal," in natural state. Such assertions have become absolutely untenable after TSCHETWERIKOFF, TIMOFEEFF-RESSOVSKY, DUBININ and his collaborators, GORDON, STURTEVANT, and others have shown that wild populations of various species of *Drosophila* carry numerous recessive mutants in heterozygous condition. Now we may go even further. Certainly more than 50 percent of wild third chromosomes in *D. pseudoobscura* carry recessive genetic variations. If other autosomes of this species behave like the third, and preliminary experiments of STURTEVANT (1937) suggest that this is the case, very few wild individuals can be free of mutational changes.

Such a situation is not as unexpected as it may seem. Indeed, the well known theory of inbreeding and heterosis advanced by G. H. SHULL, EAST, and JONES implies that most individuals of a cross-breeding species carry some deleterious recessives in heterozygous condition. Our observations constitute perhaps the best evidence on record in favor of this theory. Evidently, the "normal" viability of a species is largely a result of heterosis. This raises numerous problems regarding the mechanisms operating in wild populations to maintain these heterosis effects. The question that is most pressing is how frequently are individuals homozygous for the deleterious recessives produced in nature? This, evidently, depends upon the frequency of a given lethal or semilethal in a population. An appropriate study is under way, and its results will be reported separately.

The great frequency in wild populations of genetic factors producing slight deleterious effects has an interesting analogy in certain experimental results of TIMOFEEFF-RESSOVSKY (1935). This investigator has shown that X-ray treatment of *D. melanogaster* induces lethals, semilethals, as well as mutations with relatively minor deleterious effects, the last class of mutations being about twice as frequent as the lethals and semilethals combined. The method of the detection of these mutants used by TIMOFEEFF-RESSOVSKY was essentially the same as ours, namely observations on ratios of certain classes of flies appearing in experimental and in control cultures. The curves published by him (loc cit, fig. 3) have a striking resemblance to those represented in our figure. 2. The difference is that TIMOFEEFF-RESSOVSKY's curve for treated chromosomes has the same mode as the control curve, while our curve for the homozygotes is displaced in the minus direction compared with the curve for the heterozygotes. TIMOFEEFF-RESSOVSKY could probably obtain the same result by treating his flies with X-rays repeatedly in several consecutive generations.

## SUMMARY

1. Samples of wild populations of *D. pseudoobscura* inhabiting mountain ranges in the Death Valley region are externally homogeneous. A technique for the detection of concealed, genetic variability in the third chromosome is described (pp. 465, fig. 1).

2. Among the 849 wild third chromosomes tested, 11.9 percent carry recessive lethals and 3.1 percent semilethals. There is no sharp distinction between these classes, hence one may state that 15 percent of the wild third chromosomes contain genes that in homozygous condition destroy their carriers as an effective part of the breeding population.

3. The viability of individuals carrying two third chromosomes of different origin is defined as "normal." About 39 percent of wild third chromosomes contain factors that reduce the viability below normal, and about 2 percent factors that raise it above the average.

4. No less than 3.5 percent of the wild third chromosomes carry recessive mutant genes with visible external effects. A part of the mutants found in our experiments are allelomorphs of previously known types, and others are new.

5. A quantitatively not yet determined fraction of the wild chromosomes carries modifiers of the development rate. Both retardations and accelerations of the development are observed, the former more frequently than the latter.

6. The ten population samples studied have come from an equal number of different localities. Nevertheless, they all showed a frequency of concealed genetic variations of the same order of magnitude. This, in conjunction with similar data of STURTEVANT (1937), leads us to believe that the conditions found in our material are universal for populations of *D. pseudoobscura*.

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# THE RELATION BETWEEN SATELLITE SIZE AND NUCLEOLUS SIZE IN THREE RACES OF *SOLANUM LYCOPERSICUM*

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Received May 2, 1938

## INTRODUCTION

SHORT A chromosomes are associated with the nucleolus in most wild and cultivated tomatoes but cultivated races with long A chromosomes occur commonly. A third and new type of A chromosome, much longer than the long A, was found in the summer of 1937. It is called "very long A." Both the long A and the very long A chromosomes have increased in size by large additions to the satellite end. That the increase is chiefly due to increased satellite size can best be seen in young microspores in the resting condition. At this stage the satellite remains thick and deeply stained while the rest of the chromosome is slender and pale. The same contrast between the satellite and the rest of the chromosome is evident in somatic divisions, but in meiosis the satellite can only be distinguished from the rest of the A chromosome by the position of the attachment constriction which is at or near the base of the satellite in all three types of A chromosome. Except for satellite size, long A and short A races cannot be distinguished somatically. The chromosome size of each parent is maintained in  $F_1$  and Mendelian segregation has been shown to occur (LESLEY and LESLEY 1935).

The earlier literature on change in satellite size is discussed in the paper just referred to. DOBZHANSKY (1935) found that in *Drosophila pseudoobscura* the absolute and relative lengths of the two limbs of the Y chromosome vary, giving rise to six cytologically recognizable types of Y and that: "The morphological and physiological properties of a given strain are apparently not affected by the type of Y chromosome present in that strain." DOBZHANSKY and BOCHE (1933) suggest that the other types of Y chromosome may have been derived from the longest V-shaped type by loss of a part of a limb. In tomato the reverse is true. Both the Y chromosome in *D. pseudoobscura* and the A chromosome in tomato are associated with the nucleolus and the change in size is due to changes in amount of genetically inert material.

## ORIGIN OF "VERY LONG A" CHROMOSOME

The new type of A chromosome, very long A, appeared in the  $F_2$  from a cross between two long A races. It is probably of recent origin, since it

had never been observed before, and both parents were known to have two long A chromosomes. The pedigree of the race with very long A chromosomes (37.010) is as follows:

$$33.045.69 \times 34.030.2 \rightarrow 35.056.1 (F_1) \rightarrow 37.010 (F_2).$$

Of the 16 plants of population 37.010 which were examined for chromosome size, 10 had one long and one very long A chromosome, 5 had two long, and one, 37.010.10, had two very long A chromosomes. Since both parents, 33.045.69 and 34.030.2, were known to have had two long A chromosomes, and since 37.010 is clearly segregating for the very long A, the addition of satellite material must have occurred at some cell division, probably during meiosis, in one of the  $P_1$  parent plants.

Chromosome distribution during meiosis is about as normal in 37.010 as in short A or long A races. Unfruitfulness is also independent of chromosome size. It will be seen from table 1 that, in 37.010, a plant with one very long A chromosome may have very good pollen and be relatively fruitful as compared with long A long A plants; even 37.010.10 had several fruits each containing a few seeds. In appearance this plant was very much like 37.010.13 with two long A chromosomes, and 37.010.39 and 37.010.42, with one long and one very long A. Plant characters seemed to be determined by segregation of genes rather than by difference in chromosome size.

#### EFFECT ON POLLEN

The chromosomes of 37.010 were first examined because the whole population was very unfruitful, whereas, in other populations used for a study of male sterility, only the male-sterile plants were unfruitful. Table 1 shows that in 37.010 all plants, except male steriles (marked o), had at least one-fourth to one-half good-looking pollen in July. On September 22, the pollen was nearly all aborting at an early stage, but on October 4 several plants had much more good pollen than they had in September. The same type of variation occurred to a lesser extent in the extremely fruitful population 37.011, in which even the male-sterile plants were as fruitful as most plants capable of having much normal-looking pollen in population 37.010. The pollen abortion was probably due to the great mid-September heat in both cases. Population 37.011 was an  $F_2$  from a cross of the male-sterile plant 34.021.9 by the male-fertile 34.030.2, one of the  $P_1$  plants of the  $F_2$  population 37.010. Plant 34.021.9 was from a selfed sib of 33.045.69, the male-sterile  $P_1$  parent of 37.010.

#### SIZE OF NUCLEOLUS

Before 37.010 was found to be segregating for a very long A chromosome (figs. 1, a-b and 2, a-f), the diameter of the nucleolus in p.m.c. at pachy-

TABLE I

*A comparison of chromosome type, pollen development, and fruitfulness in populations 37.010 and 37.011.*

## Legend

*For pollen*

o = male sterile; many starch grains in mature pollen  
 — = early abortion of nearly all pollen, brownish anthers common  
 + =  $\frac{1}{2}$  to  $\frac{3}{4}$  clear, full-sized grains  
 ++ = more than  $\frac{1}{2}$  clear, full-sized grains

*For fertility*

— = decidedly unfruitful  
 +? = slightly more fruitful  
 + = possibly fruitful  
 ++ = very fruitful

| PLANT<br>NUMBER | CHROMOSOME<br>TYPE   | POLLEN<br>DATES EXAMINED |      |      | FRUITFULNESS<br>DATES EXAMINED |      |       |
|-----------------|----------------------|--------------------------|------|------|--------------------------------|------|-------|
|                 |                      | 7/19/37                  | 9/22 | 10/4 | 8/19/37                        | 9/21 | 10/30 |
| 37.010.1        | Long, Very long      | ++                       | +    | +    | —                              | +    | ++    |
| 37.010.2        | Long, Very long      | +                        | —    | +    | —                              | —?   | +     |
| 37.010.3        | Long, Very long      | +                        | —    | —    | —                              | —    | —     |
| 37.010.4        | Long Long            | +                        | —    | +    | —                              | —    | —     |
| 37.010.5        | Long, Very long      | o                        | o    | o    | —                              | —    | —     |
| 37.010.6        | Long, Very long      | +                        | —    | +    | —                              | —    | —     |
| 37.010.7        | Long, Very long      | +                        | +    | +    | +                              | ++   | ++    |
| 37.010.8        | Long Long            | o                        | o    | o    | —                              | —    | —     |
| 37.010.10       | Very long, Very long | +                        | —    | —    | —                              | —    | —*    |
| 37.010.11       | Long, Very long      | ++                       | —    | —    | —                              | —    | —     |
| 37.010.13       | Long Long            | ++                       | —    | —    | —                              | —    | +     |
| 37.010.15       | Long, Very Long      | +                        | —    | —    | +                              | +    | +     |
| 37.010.17       | Long Long            | ++                       | —    | —    | —                              | —?   | —?    |
| 37.010.26       | Long Long            | ++                       | —    | —    | +                              | —    | +     |
| 37.010.39       | Long, Very long      | ++                       | —    | —    | —                              | —    | —     |
| 37.010.42       | Long, Very long      | +                        | —    | —    | —                              | —    | —     |
| 37.010.51       | Long, Very long      | —                        | —    | —    | —                              | —    | —     |
|                 |                      | 7/2                      | 9/22 | 10/4 | 8/19                           | 9/21 | 10/4  |
| 37.011.1        | Long Long            | +                        | +    | +    | ++                             | ++   | ++†   |
| 37.011.2        | Long Long            | ++                       | ++?  | ++   | ++                             | ++   | ++    |
| 37.011.3        | Long Long            | ++                       | +    | ++   | ++                             | ++   | ++    |
| 37.011.4        | Long Long            | ++                       | +    | ++   | ++                             | ++   | ++    |
| 37.011.5        | Long Long            | o                        | o    | o    | +                              | —    | —     |

NOTE: On 9/22/37, plants 37.010—21, 23, 24, 25, 26, had abortive (—) pollen; 37.010—34, 36, 37, and 38 had better pollen but less than one-half good, and a good many abortive (—) anthers.

\* Anthers brownish even on 7/19/37.

† Except for the pollen steriles, the plants in 37.011 were all either ++ or + in fertility; they were mostly very fruitful.

tene had been measured in three diploid races with two short A and three with two long A chromosomes. The results indicated strongly that the size of the nucleolus had increased with the increase in satellite size. Similar measurements of nucleoli in the single diploid plant with two very long

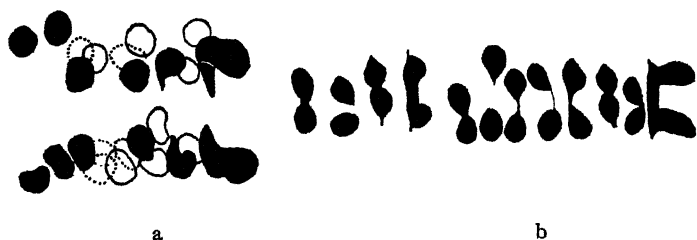


FIGURE 1.—a. Anaphase from a p.m.c. of 37.010.10 fixed in acetocarmine showing the two very long A chromosomes at the right. Both long and very long A chromosomes are usually on the periphery of the group. b. Metaphase from a p.m.c. of 37.010.10 fixed in Nawaschin's fluid.

A chromosomes (37.010.10) emphasized this conclusion (table 2). There can be no doubt that the nucleolus averages larger in 37.010.10 than in either short A short A or long A long A plants (fig. 3). It will be seen from

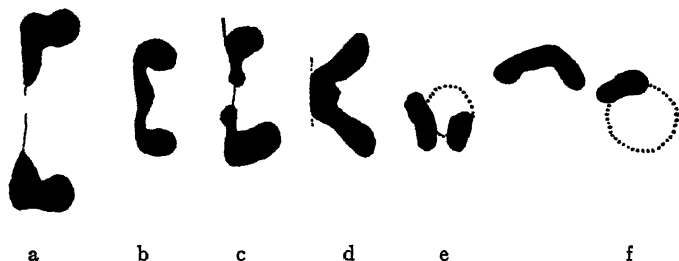


FIGURE 2.—Relative size of short, long and very long A chromosomes. All were fixed in acetocarmine. a. Very long A very long A at anaphase. b. Long A long A at metaphase. c. Very long A long A at anaphase. d. Very long A very long A at metaphase. e. Short A long A at diakinesis. f. Long A very long A at diakinesis.

table 2 that nucleolus size in 37.010.10 is very like that in a triploid with three long A chromosomes (31.014.43).

Figure 4 shows that one can easily recognize a plant having one long and one very long A chromosome from the satellite size in the tetrad stage.

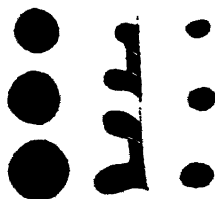


FIGURE 3.—Nucleoli, chromosomes, and satellites in short A, long A and very long A races. Nucleoli and satellites are of the average size for each type of chromosome. The upper pair of chromosomes is from short A long A; the lower from long A very long A. Chromosomes and nucleoli are from p.m.c. fixed in Nawaschin's fluid, satellites from tetrads fixed in acetocarmine.

TABLE 2  
*Measurements of the diameter of the nucleolus at pachytene.\**

| DIAMETER OF DRAWING OF NUCLEOLUS IN MM      | 5 | 5.5 | 6  | 6.5 | 7  | 7.5 | 8  | 8.5 | 9  | 9.5 |
|---|---|-----|----|-----|----|-----|----|-----|----|-----|
| A. In plants with two short A chromosomes   |   |     |    |     |    |     |    |     |    |     |
| Plant number 34.039.13                      | 0 | 5   | 34 | 7   | 2  | 0   |    |     |    |     |
| Plant number 34.001.2                       | 0 | 10  | 22 | 2   | 0  | 0   |    |     |    |     |
| Plant number 34.043.5                       | 1 | 8   | 10 | 3   | 0  | 0   |    |     |    |     |
| Totals                                      | 1 | 23  | 66 | 12  | 2  | 0   |    |     |    |     |
| Mean $d^3=213$                              |   |     |    |     |    |     |    |     |    |     |
| B. In plants with two long A chromosomes    |   |     |    |     |    |     |    |     |    |     |
| Plant number 34.065.2                       |   |     | 0  | 6   | 31 | 2   | 0  | 0   |    |     |
| Plant number 34.012.75                      |   |     | 8  | 9   | 30 | 0   | 0  | 0   |    |     |
| Plant number 34.045.69                      |   |     | 0  | 0   | 23 | 2   | 2  | 0   |    |     |
| Totals                                      |   |     | 8  | 15  | 84 | 4   | 2  | 0   |    |     |
| Mean $d^3=332$                              |   |     |    |     |    |     |    |     |    |     |
| C. Plants with two very long A chromosomes  |   |     |    |     |    |     |    |     |    |     |
| Plant number 37.010.10†                     |   |     |    |     | 7  | 13  | 39 | 6   | 8  |     |
| Plant number 37.010.10                      |   |     |    |     | 1  | 5   | 20 | 10  | 3  |     |
| Totals                                      |   |     |    |     | 8  | 18  | 59 | 16  | 11 |     |
| Mean $d^3=522$                              |   |     |    |     |    |     |    |     |    |     |
| D. A triploid with three long A chromosomes |   |     |    |     |    |     |    |     |    |     |
| Plant number 31.014.43                      |   |     |    |     | 2  | 23  | 73 | 24  | 5  |     |
| Mean $d^3=522$                              |   |     |    |     |    |     |    |     |    |     |

\* All slides were fixed in Nawaschin's fluid and stained in gentian violet. Great care must be taken that the p.m.c. do not dry out in smearing since chromosome size is much more difficult to determine with certainty if any part of the smear has become too dry. It is quite possible that this might also affect nucleolar and satellite size.

† The first set of data were taken from one slide; the second from nine others.

The diameters of the two satellites of each type were measured in this drawing and in the four microspores of a tetrad from a short A short A plant. The results follow:

| SHORT A |         | LONG A |         | VERY LONG A |         |
|---------|---------|--------|---------|-------------|---------|
| LENGTH  | BREADTH | LENGTH | BREADTH | LENGTH      | BREADTH |
| 3.0     | 2.0     | 4.0    | 2.0     | 5.0         | 2.75    |
| 2.5     | 2.0     | 3.0    | 3.0     | 4.5         | 3.00    |
| 3.0     | 2.0     |        |         |             |         |
| 2.75    | 2.0     |        |         |             |         |

The mean cube of the average of the two diameters of satellites in tetrads was 14 for short, 27 for long, and 55.7 for very long A.



The mean cube of the average diameter of satellites in young pollen was 27.7 for short A, 55.4 for long A, and 92.1 for very long A.

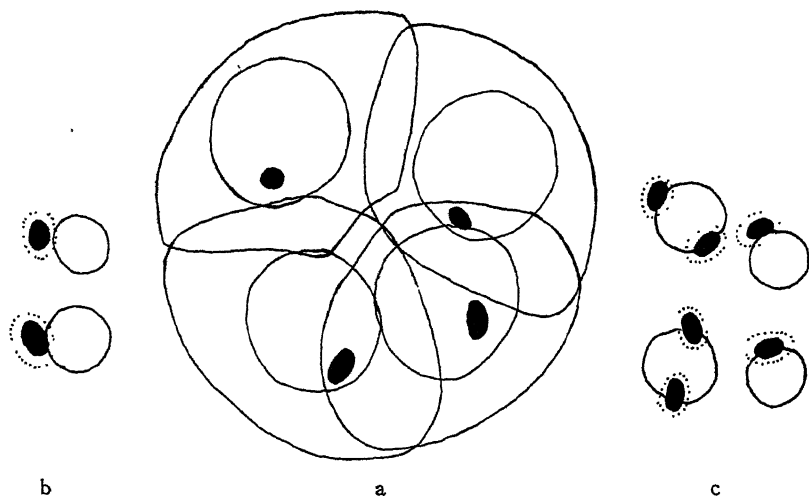


FIGURE 4.—a. Shows satellites of two sizes in the microspores of a long A very long A plant b. Shows the same and also that nucleolar size is associated with satellite size. c. Nucleoli and satellites from the four microspores of a tetrad from a triploid plant. All are from acetocarmine smears.

The satellites have in each case practically doubled in volume in the young pollen cells measured. The relation between short, long, and very long A remains similar to that found in the tetrads. The results seem to be consistent with the hypothesis that the satellite of short A has doubled in

TABLE 3

*Satellite size measured in drawings of pollen just after the rupture of the pollen mother cell wall. The length and breadth of each is given below in millimeters.*

| SHORT A |         | LONG A |         | VERY LONG A |         |
|---------|---------|--------|---------|-------------|---------|
| LENGTH  | BREADTH | LENGTH | BREADTH | LENGTH      | BREADTH |
| 3.50    | 2.50    | 4.75   | 3.00    | 5.75        | 3.75    |
| 3.25    | 2.75    | 5.00   | 2.75    | 6.00        | 3.00    |
| 3.50    | 2.50    | 4.75   | 3.00    | 5.75        | 3.25    |
| 3.75    | 2.50    | 4.75   | 3.25    | 5.75        | 3.50    |
|         |         | 4.50   | 3.25    | 5.75        | 3.00    |
|         |         | 4.25   | 2.75    | 6.00        | 3.00    |
|         |         | 4.75   | 3.00    | 5.00        | 3.50    |
|         |         | 4.75   | 2.25    | 6.00        | 3.25    |
|         |         | 4.75   | 3.00    |             |         |
|         |         | 4.50   | 3.00    |             |         |
|         |         | 4.50   | 3.25    |             |         |

size to produce long A, and that that of long A has doubled to produce very long A.

The drawings of young pollen and tetrads from which measurements of satellites were taken, were all from acetocarmine smears, but those of the nucleoli were from smears fixed in Nawaschin's fluid and stained in gentian violet. Since cells and chromosomes fixed in acetocarmine are much larger than in Nawaschin's fluid, no comparison of absolute size of nucleolus and satellite is possible. We can, however, compare the differences between nucleoli from the three types (table 2) with the differences between the satellites. Whereas the satellites approximately double in size in long A as compared with short A, and in very long A as compared with long A, the difference in nucleolar size between short and long and between long and very long is decidedly less. It is, however, greater between long and very long than between short and long. It would probably have been better to compare nucleolus and satellite size in young pollen, but unfortunately, although the satellite is easily seen in acetocarmine smears, the rest of the chromosome and the nucleolus are seldom visible, and permanent smears of tetrads were not available for all types. In a few tetrads of an acetocarmine smear of 37.010.2, a long A very long A plant, the nucleolus was visible. The nucleolus associated with the smaller satellite was decidedly smaller in each case than the nucleolus with the larger satellite.

In microspores in the resting stage which have been fixed in acetocarmine, the satellite is surrounded by a clear area. The same condition was noted at metaphase and anaphase in root tip cells fixed in Nawaschin's fluid. The clear area is probably indicative of interaction between satellite and cytoplasm during the growth of the satellite following division.

In diploids, the microspores have one satellite on the side of the nucleolus. In triploids, two microspores typically have two, the two others one satellite at the resting stage when the p.m.c. has divided to form four microcytes. Similarly, each microcyte in a tetrad of a tetraploid has typically two satellites. When the two satellites lie close together they are both associated with a single large nucleolus but when they are widely separated each may be associated with a smaller nucleolus. A nucleolus which is associated with one long A chromosome is smaller than one which is associated with two long A chromosomes and is approximately the same size as the nucleolus in a cell with one very long A chromosome. When two nucleoli are formed in one microspore in a triploid, each is of about the same size as the nucleolus in a sister cell with one A chromosome of the same type. The nucleolus in tetraploid cells with two long A chromosomes is of the same size as that in triploid cells with the same A chromosome complement. Cell size and nucleolus size do not increase proportionately.

At pachytene in pollen mother cells of 37.010.10, the two very long A

chromosomes differ from those of long A long A and short A short A races in that *each A chromosome is attached to the nucleolus at two points instead of one*. The typical figure at pachytene in 37.010.10 consists of a paired portion, two loops, and two long satellite ends (fig. 5, a-e). Pollen mother cells containing one long and one very long A have one loop (fig. 6, a-c),

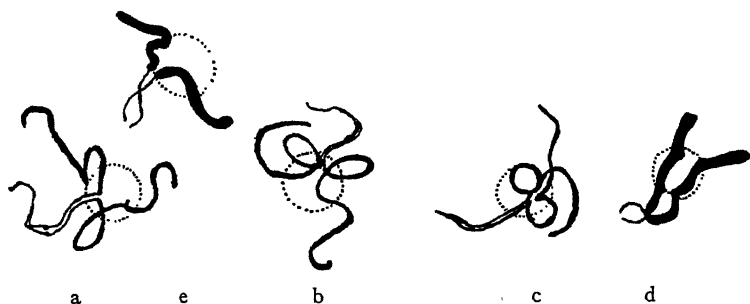


FIGURE 5.—a-c. Typical pachytene configurations in a plant with two very long A chromosomes (37.010.10); d and e show early diakinesis in 37.010.10.

those with long A long A or short A short A, none. As the chromosomes shorten the loops are gradually reduced until at late diakinesis there is often very little indication of the second attachment point. The first is also often lost in at least one A chromosome of any type at late diakinesis.

It is possible that the increase in nucleolus size in 37.010.10 is partly due to the presence of two nucleolus-forming bodies (McCLINTOCK 1934). However, in long A long A, the nucleolus-forming body is apparently no

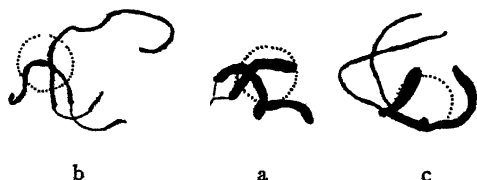


FIGURE 6.—a to c. Remnants of a loop in the very long A chromosome, none in the long A chromosome. The figures are from 37.010.1, a long A very long A plant.

longer than in short A short A and yet the nucleolus averages larger, indicating that the increased satellite material may affect the size of the nucleolus directly.

How satellite material has been added in these tomato races is not known. It seems most probable that a large block of it has, in each case, been inserted at the point of attachment to the nucleolus, since A chromosomes frequently cross at this point and the satellite ends are otherwise rarely if ever associated. A long A chromosome might have originated from a short A by insertion or terminal addition of a short A satellite and

no nucleolus-forming body; a very long A chromosome, by insertion or terminal addition of a long A satellite plus a nucleolus-forming body.

The UNIVERSITY OF CALIFORNIA Citrus Experiment Station kindly provided the materials and some of the facilities used in this study. The plants were grown by Dr. J. W. LESLEY, and I am indebted to him and to Dr. H. B. FROST for helpful suggestions.

#### SUMMARY AND CONCLUSIONS

1. Three races differing in size of the A or nucleolar chromosome have been found in tomatoes. The difference between the three types of A chromosomes (short A, long A, and very long A) is due to the addition of satellite material which appears to carry no genes.

2. At pachytene, the A chromosome is associated with the nucleolus at one point in short A and long A, at two in very long A.

3. The volume of the satellite is approximately doubled in long A as compared with short A, and in very long A as compared with long A.

4. Nucleolar size in races with two long A chromosomes averages approximately 50 percent larger than in races with two short A's. In one plant with two very long A chromosomes, the nucleoli average about 60 percent larger than in long A long A races.

5. In microspores from one p.m.c. in a triploid, a nucleolus which is associated with one long A chromosome is smaller than one which is associated with two long A chromosomes and is approximately the same size as the nucleolus in a microspore at the same stage in a cell with one very long A chromosome.

6. There is no relation between chromosome size and external plant characters, fruitfulness, or pollen abortion.

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# CHROMOSOME ABERRATIONS INDUCED BY X-RAYS

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Received June 3, 1938

## INTRODUCTION

SINCE the discovery by MULLER and STADLER that X-rays induce mutations in animals and plants, a new field has been developed in experimental genetics. This work on radiation genetics has been reviewed by MULLER (1932), HANSON (1933), OLIVER (1934), STADLER, GOOD-SPEED, GOWEN, et al. (DUGGAR 1936), STUBBE (1937), and TIMOFEEFF-RESSOVSKY (1937). The genetic results show that (1) the mutation rate increases directly with dosage, (2) the X-ray effect is not delayed or indirect, (3) there is no temperature coefficient, (4) differential susceptibility is found in different stages of development, (5) the X-rays cause translocations, inversions, and deletion of chromosome segments, (6) the induced mutations are not distributed entirely at random in the chromosomes, (7) there is no differential effect of the various wave lengths in the X-ray range, and (8) the gene string is already partially split in *Drosophila* sperm and in *Zea* pollen grains.

A direct cytological analysis of X-ray effects has confirmed some of the results obtained by genetic methods, but most of the cytological work has dealt with the nature of the chromosome rearrangements and the time of splitting of the chromonema. There is still no critical evidence regarding the relation between dosage and chromosome aberrations, the effect of temperature on chromosome susceptibility to radiation, differential susceptibility at various times during the meiotic and mitotic cycles, the mechanism involved in translocation and inversion, and the time of chromonema doubling. An analysis of X-ray effects on chromosomes of *Tradescantia* microspores has solved some of these problems.

## MATERIALS

Microspores of *Tradescantia* were used for the study of X-ray effects on chromosome behavior. The meiotic and mitotic cycle in microspore formation is well known, the chromosomes are large, and certain species flower throughout the year in the greenhouse. During the summer months the meiotic cycle from earliest prophase to the tetrad stage covers about one week, and a similar period is required for microspore development up to the time of nuclear division. The nucleus of the newly formed microspore remains in the resting stage for about five days, and is in the prophase stage for at least one day before nuclear division occurs. The length

of the meiotic and mitotic cycles is increased during the winter months and may be increased to two weeks for each cycle. All experiments were done with a clonal line of a *Tradescantia reflexa* hybrid, which has six pairs of chromosomes and one pair of fragments. Flowering stalks were cut off and kept in a glass of water during radiation and for several days to a week more while the microspores were being examined. When the microspores were to be examined for a period of several weeks after irradiation, the potted plants were subjected to X-rays.

The source of the X-rays was a Coolidge tube with a tungsten target. The line voltage was 120 at 10 ma, and the secondary voltage was 160 kv. No screen was used, and the target distance was about 75 cm. At this distance the tube delivered about 25 r per minute. The dosage used ranged from 75 to 200 r for the analysis of types of chromosome aberrations.

#### CYTOLOGICAL OBSERVATIONS

A few observations were made at meiosis, but most of the data were based on microspore chromosomes. A few hours after raying, the meiotic cells show clumping of the chromosomes and fusion of homologous chromatids. The terminal association of chromosomes is not accompanied by fragments at either the first (fig. 1<sup>1</sup>) or the second (fig. 2) meiotic division. Many sub-terminal associations are found, especially at the second meiotic anaphase, but free fragment chromosomes were not observed. Twenty-four hours after raying, many chromosome bridges and free fragments were found at anaphase of both meiotic divisions.

The mitotic division in the microspore also shows a clumping of the chromosomes shortly after irradiation. At four to six hours after raying, about half the anaphase figures show terminal or subterminal fusion of sister chromosomes (figs. 3 and 4). Occasionally there are free fragments or evidence of unequal chromatid interchange, but these are rare. In no case were fusions or interchanges found between non-homologous chromatids or chromosomes during the first seven hours after irradiation. These early fusions following X-ray treatment appear to involve the chromosome envelope, and although fragments are released by breakage at points of fusion in some figures, the fusion of sister chromatids at these stages is not of primary significance.

When moderate doses of X-rays are given to microspores the metaphase and anaphase figures can be analyzed at any time after irradiation. During the first 24 hours after raying, most of the breaks involve only one of the two chromatids (figs. 5, 6, 8, 9, and 10), but chromatid breaks have been observed as late as 72 hours after raying. Achromatic lesions also are

<sup>1</sup> Figure references are to Plates 1 and 2 unless otherwise specified.

frequent, and seem to be caused by breaks which have not released the distal ends of the chromatids (fig. 6).

The chromatid breaks may be single and release the distal end of a chromatid, or they may involve two chromatids, one from each of two chromosomes. A single break may produce an acentric fragment, or the break may be incomplete and produce an achromatic lesion (fig. 6). The double breaks may produce either reciprocal interchange of chromatids, or chromatid fusion accompanied by fused chromatid fragments. The reciprocal chromatid interchanges usually are equal or nearly equal (figs. 9 and 10), although unequal interchange of chromatid arms does occur (fig. 8). The ends of two broken chromatids may fuse to form a dicentric chromatid and release an acentric fused fragment (figs. 9 and 15). In practically all cases of chromatid fusion an acentric fragment is released. The ends of the fragment are the normal ends of the two broken chromatids, and at the point of breakage the two chromatid fragments are fused.

#### DESCRIPTION OF PLATES

Camera lucida drawings of meiotic and microspore chromosomes at various times after X-ray treatment. Acetocarmine preparations of *Tradescantia reflexa* hybrid. Magnification  $\times 900$ .

#### EXPLANATION OF PLATE I

FIGURE 1. Meiotic anaphase. Terminal fusion of chromatids. No fragments. 150 r. 3 hrs.

FIGURE 2. Second meiotic anaphase. Terminal and sub-terminal fusion of chromatids. No fragments. 150 r. 6 hrs.

FIGURE 3. Anaphase in microspore. Fusion of chromatids. 75 r. 6 hrs.

FIGURE 4. Anaphase in microspore. Terminal fusion of chromatids. Translocation between sister chromatids. 75 r. 6 hrs.

FIGURE 5. Microspore metaphase. Two chromatid breaks, and a chromosome break followed by fusion of broken ends of sister chromatids. 200 r. 12 hrs.

FIGURE 6. Microspore anaphase. Complete and incomplete chromatid breaks, and a dicentric chromosome resulting from sister chromatid fusion after a chromosome break. 100 r. 19 hrs.

FIGURE 7. Microspore metaphase. A chromosome break followed by sister chromatid fusion. 100 r. 6 hrs.

FIGURE 8. Microspore metaphase. A chromatid and a chromosome break in the same chromosome. The unequal chromatids of two chromosomes are the result of unequal reciprocal chromatid translocations. 100 r. 17 hrs.

FIGURE 9. Microspore metaphase. Chromatid exchange and chromatid fusion. Also a simple chromosome break. 75 r. 24 hrs.

FIGURE 10. Microspore metaphase. Chromatid and chromosome breaks. 100 r. 24 hrs.

FIGURE 11. Microspore metaphase. Chromatid ring formation. 75 r. 24 hrs.

FIGURE 12. Microspore—early anaphase. Chromatid and chromosome breaks. 100 r. 24 hrs.

FIGURE 13. Microspore anaphase. Dicentric chromosome and fused fragment following chromosome break. 100 r. 24 hrs.

FIGURE 14. Microspore metaphase. Complex fusion of chromosomes with no free fragments. 100 r. 28 hrs.

FIGURE 15. Microspore metaphase. Reciprocal chromatid fusion of the reverse crossover type. 180 r. 48 hrs.

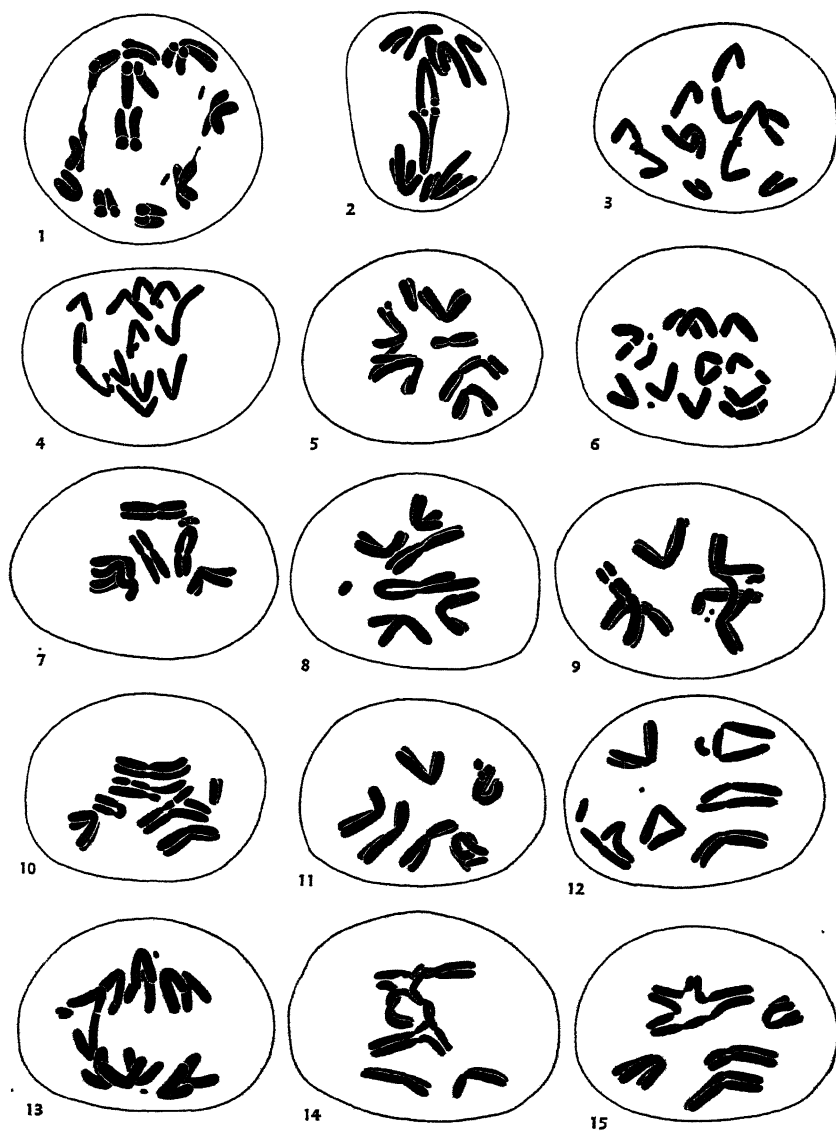


PLATE I



Few chromatid breaks are found 48 hours after raying, and none from the fourth to the ninth day. During the winter months, the microspores examined 9 or 10 days after raying were X-rayed at late stages in meiosis or very early in microspore development. Although no chromatid breaks were found between the fourth and eighth day after irradiation, a few were found on the ninth day. Most of these were in a single microspore, where two single breaks and two chromatid fusions were found at metaphase (fig. 23). A single chromatid fusion was also found at anaphase in another cell (fig. 24).

Chromosome breaks, with both chromatids broken at the same locus, were found at all times after raying the microspore. During the first 24 hours all the chromosome breaks are single. The break releases the distal end of the chromosome arm, and the broken ends of sister chromatids invariably fuse to form a U-shaped acentric fragment and a pair of sister chromatids fused at one end. The first chromosome break was observed 6 hours after raying (fig. 7). As the broken chromosome divides at anaphase, the fused ends form a bridge (figs. 6, 12, and 13). The distal ends of the broken chromatids always fuse to form a single fragment. The size of the fragment varies considerably, but no bridge has been observed without a fragment.

Single chromosome breaks are found less frequently after the second day following irradiation. At this time there is no fusion of the ends of broken chromatids, and only pairs of chromatid bridges are found. The distal fragments appear as paired rods (figs. 22, 23, and 24).

Breaks in two chromosomes may be followed by reciprocal interchange or by chromosome fusion with the release of a fragment. The reciprocal interchanges are difficult to detect, presumably because they are approximately equal, but unequal interchanges have been observed. The fusion of broken ends of different chromosomes may produce also a dicentric chromosome and a pair of chromatid fragments. Each fragment chromosome is composed of the ends of two non-homologous chromosomes fused together at the point of the break. As the dicentric chromosomes separate at anaphase, they may separate freely, or form two bridges, or interlock, depending on the amount of relational coiling between centromeres (figs. 16 and 17). Chromosome bridges are always accompanied by chromosome fragments. The size of the fragment may be no longer than the width of a chromatid or may be as long as a normal chromosome (figs. 16 and 18). Occasionally the break and fusion occur so near the centromeres that the duality of the centromeres in the dicentric chromosome can not be differentiated. The released fragment is then as long as two normal arms (figs. 18 and 20).

Broken ends in each arm of a single chromosome may reunite to form

ring chromosomes. At anaphase the ring chromosomes may separate freely, or open out into a single dicentric ring, or they may be interlocked (figs. 19, 20, and 21). Ring chromosomes induced by raying the resting microspore nucleus are always accompanied by fragments. Premeiotic irradiation has produced a ring chromosome at the microspore division with no visible fragment. Evidently the loss of a small fragment is not always lethal.

The irradiation of meiotic cells produces a high degree of microspore sterility, but some microspores do develop. These microspores, even though they include only the more viable cells, show a large proportion of breaks. Every chromosome may be broken, but if no fragments are lost, the chromosomes develop normally (fig. 24). Occasionally diploid microspores are produced after irradiation, and these also have many chromosome aberrations (fig. 26). These microspores were produced from meiotic cells which were irradiated at interphase or during the second meiotic division.

Most of the aberrations induced by X-rays are chromosome fragmentations and fusions, but other abnormalities are found occasionally. The anaphase chromosomes may not be distributed equally to the poles, and all chromosomes may pass to the same pole. Monocentric spindles are rare, and only five were observed in the thousands of anaphase figures studied (fig. 30). The centromeres of some chromosomes and chromatids seem to be inactive in chromosome orientation at metaphase and anaphase. The inactive chromosomes may be acentric fragments which have lost the centromeres by chromosome fusion (fig. 18); but the unequal distribution of daughter chromosomes to the poles and occasional lagging chromosomes at anaphase (fig. 28) suggest that a centromere may be inactivated or prevented from dividing by X-ray treatment. In one microspore the chromosomes had developed to an early metaphase stage with no visible split in the chromosomes (fig. 29).

The sequence of appearance of various types of aberrations is of interest in an analysis of the nature of breaks and fusions of chromosomes. After the terminal fusions of chromosomes are past and the more significant aberrations appear, only chromatid and chromosome breaks are observed during the first 24 hours. For example, at 17 hours after irradiation at 100 r, 21 chromatid fragments and 10 chromosome fragments were found without a single dicentric chromatid or chromosome. These and other data show that the breaks are not dependent on previous fusions.

When the resting nucleus of the microspore is irradiated, the aberrations appearing at metaphase and anaphase include dicentric chromosomes and fragments, ring chromosomes and fragments, and simple fragments. In one series of observations, made four to seven days after raying

with a dose of 200 r, there were 69 dicentric chromosomes, 11 ring chromosomes, and 11 single distal fragments. Thus about 86 percent of the fusions are between different chromosomes, and only 14 percent are between the two arms of the same chromosomes. Single breaks without fusion constituted about 12 percent of the aberrations which could be detected. In another series of observations made at corresponding times after irradiation, the proportion of single breaks was about 17 percent (table 2). The relatively small percentage of single breaks suggests that a broken end of a chromosome has a strong tendency to fuse with another broken end, and that broken ends usually reunite after a single break.

It has been assumed by a number of cytologists that an X-ray "hit" can break only a single chromonema at a given locus, and that the occurrence of chromosome breaks proves the existence of a single chromonema at the time of irradiation. But we find both chromosome and chromatid breaks in the same division figure, or even in the same chromosome, and at various times after irradiation,—from 7 to 72 hours (table 1). It seems highly improbable that the splitting of the chromosomes is so variable in different chromosomes of the same cell or that the time of the split may vary from 7 to 72 hours before the chromosomes reach the metaphase stage. The evidence seems conclusive that both chromatids may be broken at the same time by a single X-ray "hit."

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#### EXPLANATION OF PLATE 2

FIGURE 16. Microspore anaphase. Chromosome breaks followed by fusion to produce dicentric chromosomes and acentric fragments. Two types of separation—locked and free. 200 r. 8 days.

FIGURE 17. Microspore anaphase. Dicentric chromosome with crossed chromatids. 200 r. 3 days.

FIGURE 18. Microspore—early anaphase. The pair of chromatid fragments presumably released by breaks and fusions of two chromosomes very near the centromeres. 200 r. 6 days.

FIGURE 19. Microspore anaphase. Free separation of ring chromatids. 200 r. 19 days.

FIGURE 20. Microspore anaphase. Dicentric ring chromosome. Long acentric fragment released by breaks and fusion of two chromosomes at or near centromere. 180 r. 7 days.

FIGURE 21. Microspore anaphase. Locked ring chromatids. 200 r. 7 days.

FIGURE 22. Microspore anaphase. Single chromosome break which released almost entire arm of one chromosome. 100 r. 9 days.

FIGURE 23. Microspore metaphase. Chromatid breaks and fusions induced during meiosis or very early in microspore. 100 r. 9 days.

FIGURE 24. Microspore metaphase. Numerous chromosome breaks induced at meiosis. 100 r. 11 days.

FIGURE 25. Microspore anaphase. Chromatid fusion of non-sister chromatids. 200 r. 9 days.

FIGURE 26. Diploid microspore metaphase. Three dicentric and one ring chromosome. 200 r. 9 days.

FIGURE 27. Microspore anaphase. Dicentric and ring chromosomes. 1200 r. 5 days.

FIGURE 28. Microspore anaphase. Apparent inactivation of the centromere of one chromosome. 75 r. 24 hrs.

FIGURE 29. Microspore metaphase with no chromosome split. 100 r. 11 days.

FIGURE 30. Monocentric spindle. 180 r. 4 days.

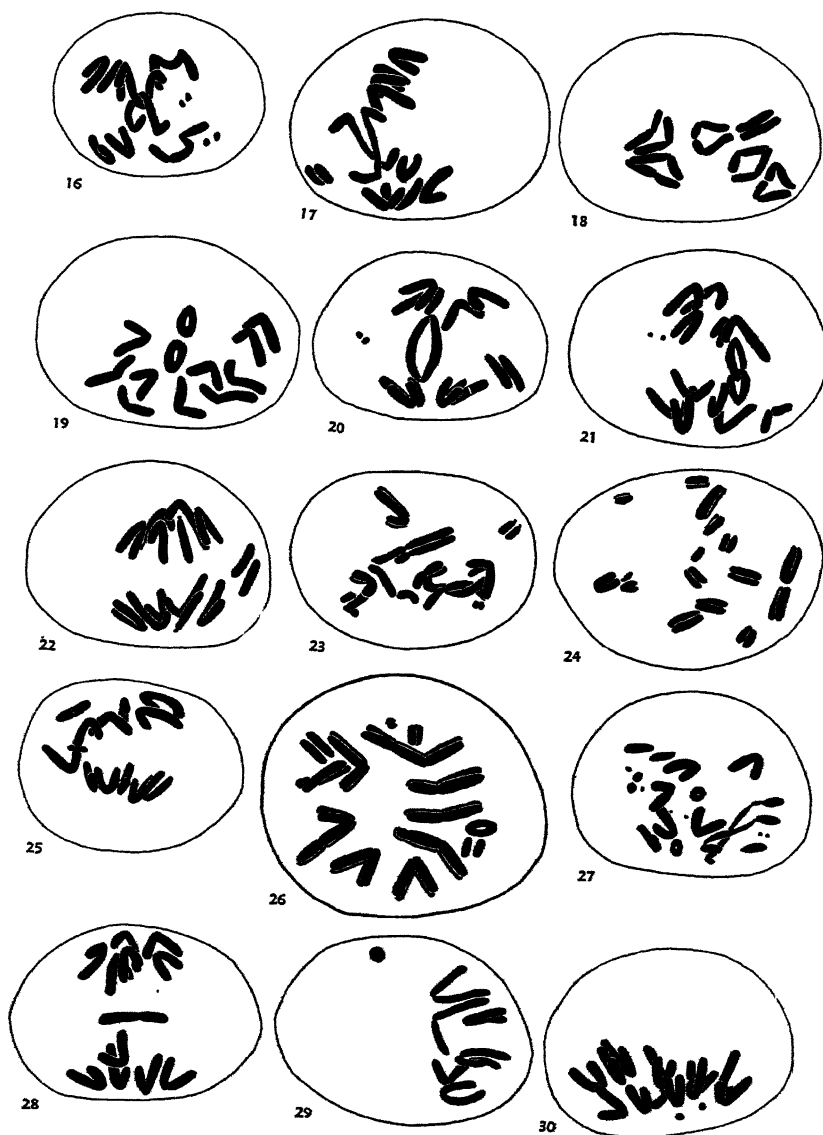


PLATE 2

TABLE 1  
*Duration of chromatid breaks.*

| HOURS AFTER<br>RAYING | 75 to 200 r         |     | CHROMOSOME<br>BREAKS | % |
|-----------------------|---------------------|-----|----------------------|---|
|                       | CHROMATID<br>BREAKS |     |                      |   |
| 5                     | 10                  | 0   | 0                    |   |
| 7                     | 22                  | 3   | 12                   |   |
| 11                    | 3                   | 1   | 25                   |   |
| 17                    | 21                  | 10  | 32                   |   |
| 24                    | 90                  | 81  | 48                   |   |
| 48                    | 15                  | 61  | 80                   |   |
| 72                    | 6                   | 41  | 87                   |   |
| 96                    | 0                   | all | 100                  |   |

If chromosome breaks and fusions occur at every locus which is hit by the X-rays, they should be distributed at random along the chromosomes. But if secondary factors are involved, such as torsion of the chromosomes or the relative positions or differential contraction of the chromosomes, then the breaks may be localized. An analysis of the position of breaks and fusions has been made, using cells which were rayed while the chromosomes were in the resting stage. The data are shown in table 2.

TABLE 2  
*Loci of chromosome breaks. 150 r. 4-7 days after raying. Length of fragment in relation to chromosome bridge or shortened arm.*

|                              | SINGLE BREAKS |    | EXCHANGE BREAKS |    |
|------------------------------|---------------|----|-----------------|----|
|                              | N             | %  | N               | %  |
| Break near centromere        | 38            | 57 | 28              | 50 |
| Break near center of arm     | 17            | 25 | 19              | 34 |
| Break near distal end of arm | 12            | 18 | 9               | 16 |

The position of the breaks and fusions was determined from the relative length of the released fragments compared with the broken chromosome arms, or the distance between centromeres in the dicentric chromosomes. In about half the aberrant chromosomes the break had occurred in the proximal third of the chromosome arm. Breaks were less frequent in the central region of the chromosome arms and still less frequent at the distal ends of the chromosomes. Simple breaks and exchange breaks show about the same frequency of distribution at the various loci.

Organisms at different stages of development show a differential susceptibility to X-rays as measured by both the mutation rate and the frequency of chromosome aberrations. An extended analysis was made with X-rayed *Tradescantia* plants to determine the types and frequencies

of chromosome aberrations at various times in the meiotic and mitotic cycles. Two series of observations were made, one with plants which had received a dose of 75 r, and the other with plants which had been subjected to 150 r. Both series showed similar results, but more data were obtained from the 150 r series. The data are shown in table 3.

TABLE 3  
*Series 17. Jan. 10.  $\times 150$  r. Greenhouse plants.*

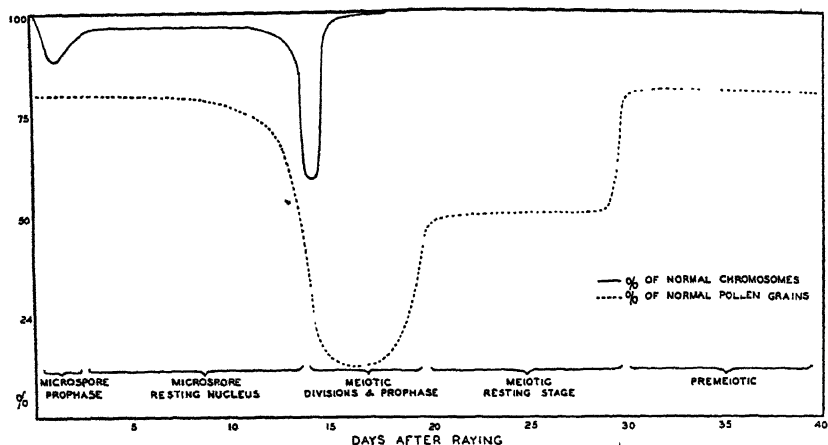
| TIME AFTER<br>RAYING | TOTAL<br>CHROMO-<br>SOMES | CHROMATID BREAKS |          | CHROMOSOME BREAKS |          | TOTAL<br>BREAKS<br>% | MIC.<br>STERIL-<br>ITY<br>% |
|----------------------|---------------------------|------------------|----------|-------------------|----------|----------------------|-----------------------------|
|                      |                           | SINGLE           | EXCHANGE | SINGLE            | EXCHANGE |                      |                             |
| 1-4 hrs.             | 318                       |                  |          |                   |          | 0.0                  | 19                          |
| 4-7                  | 936                       | 11               |          |                   |          | 1.2                  | 18                          |
| 1 day                | 450                       | 18               | 16       | 16                |          | 11.1                 | 18                          |
| 2 days               | 438                       | 3                |          | 17                | 12       | 7.3                  | 21                          |
| 3                    | 666                       | 1                |          | 4                 | 20       | 3.8                  | 18                          |
| 4                    | 612                       |                  |          | 3                 | 10       | 2.1                  | 20                          |
| 5                    | 384                       |                  |          | 3                 | 8        | 2.9                  | 23                          |
| 7                    | 354                       |                  |          |                   | 6        | 1.7                  | 20                          |
| 8                    | 288                       |                  |          |                   | 10       | 3.5                  | 22                          |
| 9                    | 372                       |                  |          | 1                 | 12       | 3.5                  | 26                          |
| 10                   | 486                       |                  |          | 3                 | 10       | 2.7                  | 26                          |
| 11                   | 528                       |                  |          | 3                 | 12       | 2.8                  | 26                          |
| 12                   | 450                       |                  |          | 5                 | 16       | 4.7                  | 28                          |
| 14                   | 108                       |                  |          | 15                | 30       | 41.7                 | 65                          |
| 15                   | 114                       |                  |          | 3                 |          | 2.6                  | 92                          |
| 16-19*               | 120                       |                  |          |                   |          | 0.0                  | 82                          |
| 19-29*               | 522                       |                  |          |                   |          | 0.0                  | 50                          |
| 29-33                | 258                       |                  |          |                   |          | 0.0                  | 20                          |

\* Fragments in one nucleate microspore.

There was an increase in chromosome aberrations up to 24-30 hours after raying, when the proportion of breaks reached 11 percent. Each chromatid or chromosome fusion was counted as two breaks, since the evidence indicates that the breaks precede the fusions. At 48-55 hours the percentage of aberrations decreases and reaches a point of stability at about 3 percent between the third and eleventh day. During this entire period the microspore fertility is normal, about 80 percent. On the twelfth day after raying, there was a slight increase in both chromosome aberrations and microspore sterility. The sterility is judged by the failure of nuclear development and microspore growth. Cells examined later showed a great increase in chromosome aberrations to over 40 percent, while the pollen sterility was also greatly increased. These microspores undoubtedly were rayed during meiosis. The pollen sterility on the 15th to 19th day was so great that few chromosome studies could be made. The high degree of microspore sterility indicates, however, that chromosome aberrations are very frequent at meiotic prophase. On the 19th day,

pollen fertility is increased to about 50 per cent and remains at this figure for about 10 days. During this period no chromosome aberrations were observed, although certain types undoubtedly are included in viable microspores. At the end of four weeks both the mitotic and meiotic cycles have been completed. The pollen fertility now becomes normal, in spite of the fact that the premeiotic cells have been X-rayed.

TABLE 4



The relation between chromosome aberrations, microspore sterility, and the stage of nuclear development, is shown in table 4. The susceptibility of the chromosomes to X-ray treatment is greatest at meiosis and presumably at meiotic prophase. Since all the chromosomes found in a tetrad of resting microspores are already differentiated at late pachytene of meiosis, it appears that the meiotic chromosomes are much more susceptible to X-ray breakage than the chromosomes in the resting nuclei of the microspores. In view of the selected sample of microspores resulting from X-rayed meiotic cells, it seems probable that the chromosomes at meiosis are at least ten times as susceptible as chromosomes at the resting stage in the microspore. The prophase stage of mitosis is more susceptible to X-rays than the resting stage, but at prophase about half the breaks are chromatid breaks, while X-rayed resting nuclei show only chromosome breaks at metaphase and anaphase. However, the mitotic prophase stage is about twice as susceptible to X-ray treatment as the mitotic resting stage.

#### THE EFFECT OF TEMPERATURE ON CHROMOSOME SUSCEPTIBILITY TO X-RAYS

In both plants and animals the mutation rate after X-raying is independent of the temperature at the time of irradiation. We have X-rayed

*Tradescantia* microspores at various temperatures to determine if temperature at the time of radiation has any effect on frequency of chromosome aberrations. Flower stalks of *Tradescantia* were placed in thermos bottles containing water at different temperatures. The cuttings were placed in the thermos bottles about half an hour before raying and kept in the bottles during irradiation and for one hour after raying. They were then placed in water at room temperature and examined each day for about a week. Two series of observations were made, one irradiated with a dose of 100 r at temperatures of 6° and 40°C, respectively, and the other subjected to temperatures of 7°, 25°, and 37°C and X-rayed with 200 r. The first series showed no significant difference in percentage of abnormalities at the two temperatures. Those irradiated at 6°C showed chromosome aberrations in 20 percent of the microspores, while those irradiated at 40°C showed chromosome aberrations in 19 percent of the microspores. Similar results were obtained in the second series. The data are shown in table 5. The microspores rayed at 37°C do show a higher average percentage of cells with chromosome breaks, but in view of the great variability found on different days, the differences in aberrations at different temperatures are not significant. Evidently there is no temperature coefficient for X-ray induced chromosome aberrations.

TABLE 5

*X-ray effects at different temperatures. Series 8. X200 r. Oct. 18, 1937. Tradescantia microspores.*

| DAYS<br>AFTER<br>RAYING | AT 7°C |       | AT 25°C |       | AT 37°C |       | COMBINED |       | MIC.<br>STE-<br>RILITY |
|-------------------------|--------|-------|---------|-------|---------|-------|----------|-------|------------------------|
|                         | TOTAL  | % BR. | TOTAL   | % BR. | TOTAL   | % BR. | TOTAL    | % BR. | %                      |
| 1                       | 148    | 59    | 132     | 65    | 111     | 43    | 391      | 60    | 25                     |
| 2                       | 103    | 26    | 153     | 17    | 132     | 11    | 388      | 18    | 25                     |
| 3                       | 63     | 21    | 140     | 9     | 66      | 8     | 269      | 12    | 26                     |
| 4                       | 57     | 23    | 160     | 10    | 63      | 10    | 280      | 12    | 19                     |
| 5                       | 78     | 12    | 170     | 17    | 25      | 24    | 273      | 16    | 20                     |
| 7                       | 142    | 18    | 47      | 19    | 143     | 14    | 332      | 17    | 51                     |
| 8                       | 56     | 30    | 48      | 23    | 135     | 50    | 239      | 40    | 53                     |
| 9                       | 76     | 46    | 211     | 71    | 137     | 90    | 424      | 73    | 60                     |
| Total                   | 723    | 31    | 1070    | 32    | 812     | 36    | 2605     | 33    |                        |

The percentage of breaks based on number of division figures with breaks or fusions in one or more chromosomes.

The temperature experiments were conducted in October, and the microspore cycle at this time is about one week, as compared with about two weeks in January (cf. table 3). It will be noted that on a cell basis the abnormalities induced at meiosis are only slightly greater than those at microspore mitotic prophase, but the increase in pollen sterility a week after irradiation prevents an accurate comparison.



THE RELATION BETWEEN X-RAY DOSAGE AND  
CHROMOSOME ABERRATIONS

In both plants and animals the mutation rate induced by X-rays is directly proportional to the dose. From this relationship it is concluded that single "hits" are responsible for the mutations. The accuracy with which chromosome aberrations can be analyzed in *Tradescantia* microspores makes possible a critical analysis of the relation between X-ray dosage and the percentage of chromosome aberrations.

In the first series of experiments, flowering stalks of *Tradescantia* were subjected to X-ray doses of 150, 300, 600, and 1200 r. The only varying factor in the treatment was the length of time necessary for giving the respective doses. The cytological observations were made on the third and fifth day after raying. A dicentric chromosome was classed as two breaks, as were the ring chromosomes. Since 80-90 percent of all visible aberrations are chromosome fusions accompanied by fragments, it makes little difference whether we class such aberrations as single or double breaks.

A second series of buds was subjected to X-ray doses of 100, 200, 400, and 800 roentgens. The combined data from both series are shown in table 6. The log of the dosage plotted against the log of the percentage of aberrations gives a straight line, and leads to the derivation of an equation for the relationship between dosage intensity and percentage of chromosome breaks.

$$\%B = (Ir/80)^{1.5}.$$

TABLE 6  
*Relation of breaks and X-ray dose.*

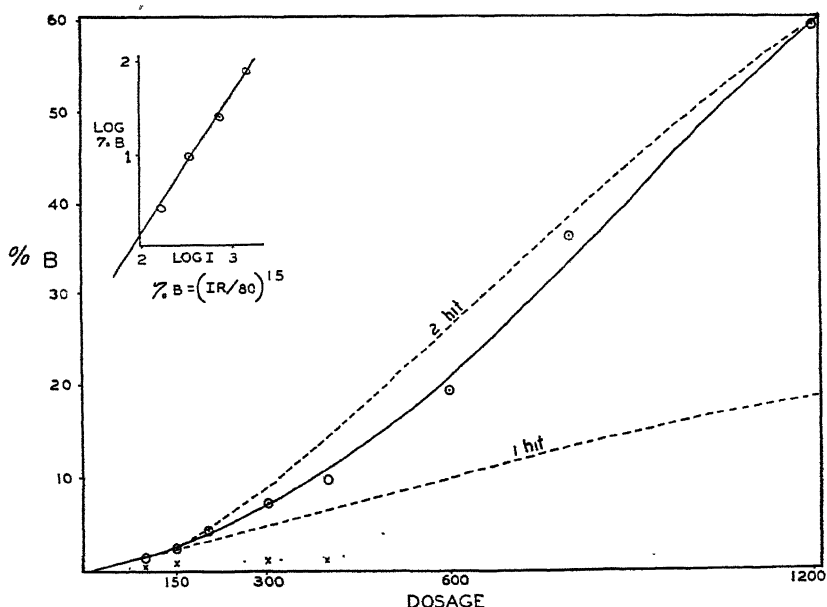
| DOSAGE | TOTAL | BREAKS | % BREAKS | (Ir/80) <sup>1.5</sup> % |
|--------|-------|--------|----------|--------------------------|
| 100 r  | 2538  | 40     | 1.6      | 1.4                      |
| 150 r  | 1896  | 48     | 2.5      | 2.6                      |
| 200 r  | 1476  | 70     | 4.7      | 4.0                      |
| 300 r  | 1626  | 120    | 7.4      | 7.3                      |
| 400 r  | 3384  | 332    | 9.8      | 11.2                     |
| 600 r  | 1446  | 275    | 19.0     | 20.4                     |
| 800 r  | 2214  | 796    | 35.9     | 31.6                     |
| 1200 r | 1086  | 644    | 59.3     | 58.0                     |

It is clear that there is no simple relationship between dosage and percentage of chromosome aberrations. Data obtained from several series of observations show that the proportion of single breaks increases directly with increased dosage, but since the single and double breaks are difficult to differentiate in the complex figures induced by higher dosages, the relationship is not completely established. For the double breaks, those which result in dicentric and ring chromosomes, the percentage of breaks increases in geometric proportion to the dosage.

The relation between dosage and chromosome breaks suggests that the double breaks are caused by independent X-ray hits. A number of investigators working with X-ray effects have devised methods for determining whether one, two, or more hits are necessary to produce a given effect. According to WYCKOFF and RIVERS (1930), if one hit is necessary to kill, the survival ratio is  $e^{-an}$ , where  $a$  is the probability than an electron will

## DESCRIPTION OF TABLE 7

The relation between dosage and chromosome aberration compared with theoretical curves based on equations for 1 hit ( $\%B = 1 - e^{-an}$ ) and 2 hits [ $\%B = 1 - e^{-an} (1 + an)$ ] reactions. Single breaks tend to occur in direct proportion to dosage.



hit the object and  $n$  is the number of electrons shot at the object. If two hits are necessary to produce the effect, the survival ratio is  $e^{-an}(1+an)$ . Applying these formulae to our data, we have determined the theoretical curve for chromosome aberrations at various dosages assuming that one hit is effective ( $\%B = 1 - e^{-an}$ ), and that two hits are necessary [ $\%B = 1 - e^{-an} (1 + an)$ ]. We have, in each case, taken an arbitrary value of  $an$  which will give the observed percentage of aberrations at 150 r. The theoretical curves and the observed values are shown in table 7. It is evident that the observed values approach the theoretical curve based on the assumption that two hits are necessary to break two chromosomes or chromosome arms, although it is possible that some of the dicentric and ring chromosomes are produced by a single hit. The single breaks are

evidently caused by single hits, even though the chromosome may be split into two chromatids at the time of breakage.

#### DISCUSSION

Most of the earlier analyses of chromosome aberrations induced by X-rays were based on the assumption that a single hit was so localized in its effect that only a single chromatid could be broken at a given locus. CARLSON (1937), working with irradiated somatic cells of *Chortophaga*, finds that an X-ray hit can break one or both of the two sister chromatids. KAUFMANN (1937) finds complex chromosome rearrangements following X-radiation of *Drosophila* sperm and concludes that sister chromatids can be broken simultaneously at the same locus by secondary effects of a single hit. This cytological work is in accord with the genetic results of PATTERSON (1933) and MOORE (1934).

The fact that both chromosome and chromatid breaks occur at metaphase and anaphase from 7 to 72 hours after raying the *Tradescantia* microspores, clearly indicates that a single hit can break one or both chromatids at the same locus. Chromosome and chromatid breaks often occur in the same cell, and may occur in the same chromosome. It seems highly improbable that the time of splitting of the chromosome varies from 7 to 72 hours before the chromosomes reach the metaphase stage. The proportion of chromosome aberrations produced by different amounts of irradiation also indicates that some of the fusions may be caused by a single hit which breaks two adjacent chromosomes.

Most of the X-ray tests show chromatid breaks when the nucleus is irradiated in the prophase stage, and chromosome breaks when rayed in the resting stage (RILEY 1936). However, MATHER (1937) does find only chromatid breaks for 160 hours after irradiation of *Allium* microspores, and we find a few chromatid breaks in *Tradescantia* microspores which were irradiated at meiosis or at the beginning of microspore development. In both *Drosophila* (PATTERSON 1933, MOORE 1934) and *Zea* (STADLER and SPRAGUE 1936) the genetic results indicate that irradiation of gametes produced both chromosome and chromatid mutations. If, as Mather admits, chromatid breaks are found in irradiated male gametes, it is probable that all the chromosomes are split, but many hits involve both chromatids at the same locus. The greater percentage of fractional deficiencies induced in *Zea* by ultra-violet as compared with X-ray effects (STADLER and SPRAGUE 1936) can be attributed to greater localization of ultra-violet effects. The X-rays may break both chromatids in the irradiated gametes, while ultra-violet rays usually break only one of the two chromatids. If chromosome breaks are dependent on the wave length of the radiation and the proximity of the chromatids, the occurrence of

chromosome breaks at the resting stage does not prove that the chromosome is single at this stage of nuclear development. The cytological studies of HUSKINS and HUNTER (1935), KAUFMANN (1937), CARLSON (1937), and MATHER (1937), all show that the chromosome is split early in the resting stage. The split may occur earlier, as NEBEL (1937) maintains, although the studies based on direct observation of the duality of the chromosome are not so critical.

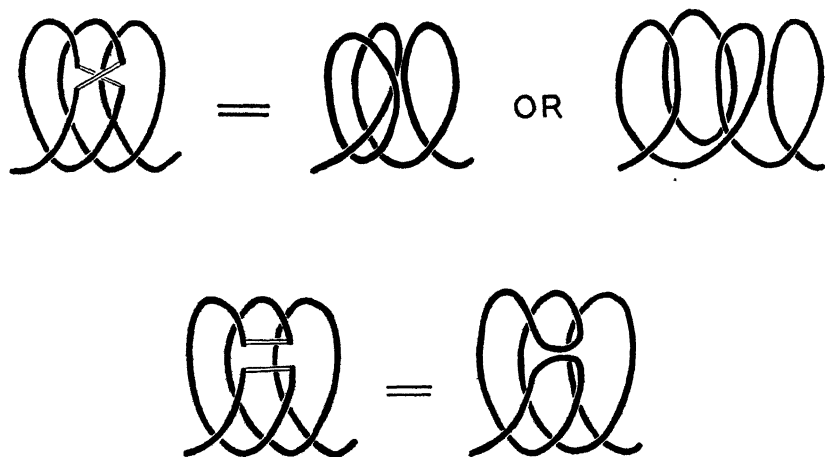
Chromosome breaks and fusions have been attributed to two different mechanisms, fusions followed by breaks, and breaks followed by a fusion of broken ends. The behavior of irradiated *Tradescantia* chromosomes strongly supports the second hypothesis. While it is true that the earliest induced aberrations are chromosome fusions, these are seldom accompanied by chromosome fragments and are of little significance in the production of permanent chromosome aberrations. The conclusions based on these temporary fusions (MARSHAK 1937) are not valid so far as permanent X-ray effects are concerned (MARQUARDT 1937 and WHITE 1937). These primary fusions are induced also by heat (SAX 1937) and by age (BARBER 1938).

All breaks during the first 24 hours after irradiation of *Tradescantia* microspores are single chromatid and chromosome breaks. Irradiation of the resting nuclei also produces single breaks, although fusions are much more frequent at this stage. These single chromatid and chromosome breaks can not be attributed to fusions followed by breaks. The evidence that most of the fusions between different chromosomes are dependent on two adjacent hits also indicates that the breaks occur first, followed by fusion of broken ends (cf. table 7).

The mutation rate is directly proportional to the dosage of X-rays, while the chromosome aberrations show a geometric increase with increased dosage. If mutation is associated with chromosome aberration, one might expect the dosage relationships of mutation and chromosome aberration to be similar. But more than 80 percent of the chromosome aberrations which can be detected involve fusions between different chromosomes, or between different arms of the same chromosome. These aberrations, as well as the simple deficiencies, will tend to be eliminated in successive cell generations, and most of the aberrations which survive will be reciprocal translocations, inversions, and small deficiencies. The small inversions and deficiencies can be induced by the effects of a single X-ray hit.

If mutations are caused by chromosome rearrangements, as several geneticists have suggested (GOLDSCHMIDT 1938), most of the structural changes must involve small aberrations which are induced by single hits. The internal structure of the chromosome offers a clue to the possible

mechanism of such changes. The chromosomes are in the form of relaxed coiled chromonemata during the resting stage. At early prophase, new minor spirals develop as the relic coils disappear. The gyres of the new minor spirals increase in size and decrease in number, so that at metaphase there are 20 to 25 coils per chromosome in the *Tradescantia* microspore (SAX and SAX 1936). A single X-ray hit can break two adjacent gyres, and the reunion of broken ends in new associations will produce small deficiencies and inversions. This mechanism is essentially the same as HUSTED (1937) finds occurring spontaneously in *Pancreaticum*. The diagrams (textfig. 1) showing these structural changes are based on HUSTED's illustrations.



TEXTFIGURE 1. Postulated mechanism for production of small deficiencies and inversions. Breaks in two adjacent gyres of the coiled chromonema, followed by a criss-cross reunion, will produce a ring deletion which is either locked around the chromonema or is free. Breaks in two adjacent gyres followed by reunion of adjacent ends will lead to a small inversion.

The size of the deficiency or inversion will depend upon the diameter of the chromonematic spiral. The diameter of the gyre will vary at different periods in the mitotic cycle. At earliest prophase, two types of spirals are found,—large relic spirals and the new minor spirals of very small diameter. At metaphase, the deletion or inversion of a gyre would involve about 4 or 5 percent of the length of the chromonema in a single *Tradescantia* chromosome. Fragments at least this small are found occasionally in irradiated microspores (figs. 21 and 27). If a similar mechanism be postulated for “molecular spirals” (DARLINGTON 1937), the aberrations could not be detected cytologically and might be considered as chemical changes in the gene.

The differential susceptibility of nuclei at different stages of development is common for both X-ray-induced mutation and induced chromo-

some aberrations (MARSHAK 1935). This differential susceptibility has been attributed to differences in pH (ZIRKLE 1936), to water content (GUSTAFSSON 1937), and to differences in amount of chromatin around the gene string (MARSHAK 1935). The differential susceptibility of meiotic and mitotic nuclei is difficult to reconcile with differences in pH involving the isoelectric point during each of the nuclear cycles. STADLER finds no differential mutation rate in X-rayed seeds which differ in water content, and GUSTAFSSON's observations on chromosome fusions may be attributed to the initiation of prophase stages induced by the absorbed water. The chromatin around the gene string certainly could not serve as an insulation against X-ray hits, although it might reduce the flexibility of the gene string so that broken ends could not fuse in new associations. As GOOD-SPEED has suggested, the cellular activity seems to play a part in X-ray susceptibility. As applied to the chromosomes, it appears that the period of greatest sensitivity to irradiation is correlated with the greatest activity in the coiling mechanism, both minor coiling and relational coiling. At this time the chromosomes appear to be under torsional strain, and some of the breaks will be prevented from rejoining in the original position, and adjacent breaks in adjacent chromatids or chromosomes will join in new associations (CATCHESIDE 1936). The great susceptibility of meiotic prophase chromosomes can be attributed to relational coiling of both chromatids and chromosomes and the greatly increased length of the chromonemata which would provide greater opportunity for union of breaks in adjacent chromosomes. Gross chromosome aberrations would not be expected frequently in the metaphase chromosomes, owing to closely coiled spirals, or in the resting stage where the chromosomes are not under much torsional strain. The distribution of X-ray-induced mutations is not at random in the chromosomes of *Drosophila* (GOWEN and GAY 1933), and chromosome breaks are not at random in *Crepis* (LEWITSKY and SIZOVA 1935), or in *Tradescantia*. The concentration of breaks in the proximal half of the chromosome arms may be associated with greater mechanical stress in that region. The differential susceptibility of different stages of nuclear development and of different chromosome loci indicates that permanent breaks and fusions are, in part, dependent upon secondary factors which are effective during irradiation.

Various attempts have been made to determine the size or diameter of the sensitive volume of the gene string by radiation-genetic and cytological methods. The number of electrons from the X-ray source which strike a nucleus or chromosome can be calculated approximately from physical data. The number of effective hits is measured by the mutation frequency or the frequency of chromosome aberrations. From these data the size or diameter of the essential part of the gene string is determined.

Assuming that the gene is spherical, GOWEN and GAY (1933) found the diameter of the sensitive volume to be  $0.01\mu$ . MARSHAK (1937), working with plants, estimates the diameter of the gene string to be  $0.001\mu$ , while HASKINS and ENZMANN (1938) calculate the diameter of the sensitive volume as  $0.014\mu$ . At best, these determinations are only rough approximations, and are subject to other errors because it is assumed that every hit in the sensitive region of the gene string produces a chromosome break or mutation, regardless of the state of development of the nucleus. However, the use of these measurements of sensitive volume is of some interest as applied to chromosome breaks in irradiated *Tradescantia*. Using MARSHAK's calculation of the number of electrons which strike a given nuclear area per roentgen of dosage, we can estimate the theoretical number of X-ray hits striking the chromosomes of *Tradescantia*. The total length of the haploid gene string in the six chromosomes of *Tradescantia* is approximately  $480\mu$ . If the diameter of the essential part of the gene string is  $0.001\mu$ , as estimated by MARSHAK, we should expect 1.5 hits per cell at 150 r, but if the gene string has a diameter of  $0.01$ , as calculated by GOWEN and GAY, we should expect 15 hits in the chromosomes of a single microspore. The actual number of breaks induced by 150 r at the time of greatest sensitivity in meiosis is about 3; at mitotic prophase, about 0.7; and at the resting stage of the microspore nucleus, about 0.2 per cell. If the maximum number of breaks is a measure of hits, the diameter of the actual gene string of *Tradescantia* chromosomes is between  $0.01$  and  $0.001\mu$ . But if any part of the visible chromonema is hit by an electron, the secondary effects could certainly spread far enough to cause breaks in the gene string, since a single "hit" can break two chromatids which are at least  $0.1\mu$  apart. Since the chromosomes are visible at the stage of greatest elongation at leptotene and early pachytene, we can take the diameter of a visible chromonema as about  $0.1\mu$ . Calculated on this basis, the total number of hits in all chromosomes of a microspore would be 150 at a dosage of 150 r. The relatively great X-ray intensities used to induce mutations in plants and animals also indicate that few X-ray hits are effective in inducing mutations.

The cytological evidence indicates that many of the breaks induced by X-ray hits do not lead to permanent aberrations. The fact that ten times as many aberrations are induced at meiotic prophase as are induced at the microspore resting stage with the same dosage, suggests that most of the potential breaks induced at the resting stage result in no change in chromosome morphology. The chromosome fusions are dependent on simultaneous breaks in closely adjacent chromosomes, while simple breaks and terminal deletions would be expected at any locus, and yet more than 80 percent of the visible aberrations are chromosome fusions. This rela-

tionship also suggests that most breaks are temporary. In addition, we have the evidence from direct observation. Achromatic lesions are frequent, and these appear to be induced by partial breakage or by a reunion of broken ends. The evidence from pollen sterility shows that few of the X-ray hits produce haploid lethals. Evidently few of the hits on the gene string and its surrounding chromatin are effective in producing either permanent chromosome aberrations or mutations.

In many respects the X-ray-induced breaks resemble the spontaneous breaks occurring at the time of crossing over. They occur most frequently at the time of differentiation of sister chromatids; broken ends usually reunite with other broken ends; the distribution of breaks which lead to aberrations is not at random in the chromosome; and there is no evidence that the break is the direct cause of mutation.

The fact that there is no temperature coefficient for X-ray-induced chromosome aberrations or mutations indicates that the X-ray effect is immediate and is not due to delayed chemical reactions. There is no evidence for any delay in either the breaking of chromosomes or the production of mutations following irradiation.

It is well known that broken ends of chromosomes have a strong tendency to fuse with other broken ends, and that the normal ends of chromosomes have characteristics not found at interstitial loci. Permanent fusion of normal chromosome ends does not occur even in irradiated cells. In some cases a break in a chromosome is followed by the fusion of the ends of adjacent broken chromatids. Breaks in inversion bridges at meiosis appear to produce a terminal fusion of sister chromatids at the break, so that a single bridge is formed at the following mitotic division in the microspore (SAX 1937). In the X-rayed microspores, such fusions occur when the chromosome is irradiated at late prophase when the chromatids are visibly differentiated; but no such fusion occurs if the chromosomes are irradiated during the resting stage. At this time, they usually react to irradiation as though there were only a single chromonema, and when the chromatids become differentiated and are analyzed at metaphase, there is no fusion of broken ends of adjacent sister chromatids (figs. 12 and 22). This behavior indicates that broken ends of chromosomes induced by X-rays may function as normal ends and separate freely without fusion and bridge formation. It is not clear how these results can be reconciled with earlier observations.

A comparison of X-ray-induced mutations and induced chromosome aberrations shows great similarity of the reactions. Both show an immediate effect of irradiation; there is no temperature coefficient for X-ray effects; there is differential susceptibility to irradiation; and there is some evidence of a differential effect at various loci of a single chromo-



some. The discrepancy in dosage-response relations can be attributed to the elimination of most of the gross chromosome aberrations. The X-rays are not unique in their effects on mutation and chromosome aberrations, because both changes are produced simultaneously by age, or heat, or genetic factors (SAX 1937).

The similarity in chromosome aberration and mutation response to irradiation; the simultaneous production of both chromosome aberration and mutations by X-rays, heat, and age; and the fact that many of the X-ray hits produce neither chromosome aberrations nor mutations, suggests that mutations are produced by structural changes in the chromosomes. Mutations frequently are associated with chromosome aberrations, and several geneticists have suggested that all mutations are caused by deletions or chromosome rearrangement (GOLDSCHMIDT 1938). SOKOLOW (1937) believes that all mutations are caused by position effects, some of which involve only a few bands in the salivary chromosome of *Drosophila*. DEMEREC (1937) finds that many of the X-ray-induced lethals in *Drosophila* are associated with chromosome deficiencies; but for spontaneous lethals and for visible mutations, no corresponding chromosome aberrations could be detected in the salivary gland chromosomes. Moreover, many known translocations and inversions in both plants and animals are not associated with any phenotypic differentiation. If mutations are caused only by chromosome aberrations, many of the changes must be of a submicroscopic order, and the distinction between gene changes and position effect becomes arbitrary.

#### SUMMARY

An analysis of X-ray induced chromosome aberrations in *Tradescantia* microspores has shown that:—

1. The first recognizable aberrations are fusions of sister chromatids with no acentric fragments. The more significant visible aberrations which appear later include terminal deletions of chromatids and chromosomes, chromatid and chromosome fusions accompanied by acentric fragments, and reciprocal translocations.
2. Chromosome rearrangements are caused by breaks followed by fusion of broken ends.
3. The effects of a single X-ray "hit" can break one or both sister chromatids, and may break two adjacent chromosomes.
4. Most of the X-ray "hits" cause no permanent breaks in the chromosome and have no lethal effect on the male gametophyte. In many respects the induced breaks are similar to natural breaks occurring at the time of crossing over.

5. The chromosome is split early in the resting stage of the nucleus, and may be split earlier.

6. The percentage of gross chromosome aberrations increases geometrically with increased X-ray dosage, indicating that most of the chromosome fusions are dependent upon two independent adjacent hits. Most of these aberrations are eliminated in a few cell generations.

7. There is no temperature coefficient for X-ray induced chromosome aberrations, indicating that the X-ray effect is not caused by a secondary chemical reaction.

8. There is considerable differential susceptibility to irradiation at different periods in the meiotic and mitotic cycles. The greatest frequency of chromosome aberrations is associated with greatest chromosome activity,—at meiotic and mitotic prophase.

9. Chromosome breaks do not occur at random in the chromosome.

10. The relation between irradiation and chromosome aberration is similar to the relation between irradiation and mutation. Small inversions and deletions induced by a single "hit" can be attributed to breaks and fusions in adjacent coiled chromonemata.

This work was supported, in part, by a grant from the National Research Council Committee on Radiation. The writer is indebted also to Dr. W. J. CROZIER for the use of the X-ray equipment, to Dr. E. V. ENZMANN for raising the *Tradescantia* buds, and to Margery Poole for technical assistance.

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# SOMATIC VARIATION OF CHROMOSOME NUMBERS IN HYBRID WHEATS<sup>1</sup>

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Received June 2, 1938

**D**URING an examination of meiosis in 336 plants of 50 *vulgare*-like lines, derived from interspecific crosses involving four varieties of *Triticum vulgare* and *T. durum* var. Iumillo, pollen mother-cells with aberrant chromosome numbers were found in plants with 39, 40, 41 and 42 chromosomes.

## MATERIAL AND METHODS

The 50 lines used in this study were obtained from Dr. R. F. PETERSON, Dominion Rust Research Laboratory, Winnipeg, Manitoba. The parental varieties were: *T. vulgare* ( $2n=42$ ) var's. Marquis, Marquillo (derived from a Marquis  $\times$  Iumillo cross), Hope (derived from a Marquis  $\times$  Yaroslav Emmer cross), and R.L. 729 (derived from a Pentad *durum*  $\times$  Marquis cross); *T. durum* var. Iumillo ( $2n=28$ ).

One anther, only, was used in the preparation of each slide, and the observations were made *before* and after the smear slides were made permanent.

## OBSERVATIONS

From the results summarized in table 1, it is seen that aberrant pollen mother-cells were found in one or more plants of all crosses studied, and in all generations recorded with the exception of F<sub>7</sub> Iumillo  $\times$  R.L. 729.

TABLE 1  
*The distribution of the aberrant pollen mother-cells.*

| MATERIAL                                  | NUMBER OF FAMILIES WITH<br>ABERRANT PLANTS/TOTAL<br>FAMILIES STUDIED | NUMBER OF PLANTS WITH<br>ABERRANT PMC/TOTAL<br>PLANTS STUDIED |
|---|--|---|
| Marquis $\times$ Iumillo F <sub>7</sub>   | 1/ 9   | 1/ 50   |
| Iumillo $\times$ Hope F <sub>7</sub>      | 5/12   | 8/ 82   |
| Iumillo $\times$ Hope F <sub>6</sub>      | 1/ 5   | 2/ 28   |
| Iumillo $\times$ R.L. 729 F <sub>7</sub>  | 0/ 4   | 0/ 28   |
| Iumillo $\times$ R.L. 729 F <sub>6</sub>  | 4/ 7   | 4/ 46   |
| Marquillo $\times$ Iumillo F <sub>1</sub> | 1/13   | 1/102   |
| Totals                                    | 12/50  | 16/336  |

<sup>1</sup> Contribution No. 108, Cereal Division, Central Experimental Farm, Department of Agriculture, Ottawa, Canada.

The frequencies of plants with aberrant pollen mother-cells in the various crosses were: Marquillo  $\times$  Iumillo, 0.98 percent; Marquis  $\times$  Iumillo, 2.00 percent; Iumillo  $\times$  R.L. 729, 5.45 percent; and Iumillo  $\times$  Hope, 9.09 percent (*cf.* table 1). Aberrant pollen mother-cells were found in six of the 140 plants with 42 chromosomes (4.2%), seven of the 98 plants with 41 chromosomes (7.1%), two of the 50 plants with 40 chromosomes (4.0%), and one of the 27 plants with 39 chromosomes (3.7%).

Seventy-six aberrant pollen mother-cells were observed among the 505 pollen mother-cells examined in 16 plants distributed among 12 families. The number of aberrant pollen mother-cells recorded in any one plant varied from one to 20. Their chromosome complements varied from 15 to 61, and the number of bivalent chromosomes varied from three to 29. The data are given in table 2. Details of the chromosome number and arrangement in aberrant pollen mother-cells of two plants are given in tables 3 and 4. Typical aberrant pollen mother-cells are illustrated and for comparison, four "normal" (2n) pollen mother-cells from plants with 39, 40, 41 and 42 chromosomes are also included (Plate I, figures 1-11).

#### DISCUSSION

HOLLINGSHEAD (1932) reported the rare occurrence of one or a small group of pollen mother-cells with less than the normal number of chromosomes in Marquillo and in a hybrid of *T. vulgare* var. Garnet with an unknown variety.

KATTERMAN (1933) described the occurrence of such hypoploid pollen mother-cells in wheat-rye hybrids. His paper also included a review of the literature on cytomixis, to which phenomenon he ascribed the abnormali-

#### DESCRIPTION OF PLATE I

Photomicrographs of pollen mother-cells in *vulgare*-like derivatives of pentaploid wheat hybrids. Aceto-carmin preparations.  $\times 540$ .

FIGURE 1. Normal cell in a 39-chromosome plant. Nineteen bivalents and one univalent.

FIGURE 2. Aberrant cell in the same plant. Six bivalents and eight univalents.

FIGURE 3. Normal cell in a 40-chromosome plant. Nineteen bivalents and two univalents.

FIGURE 4. Aberrant cell in a 40-chromosome plant. Ten bivalents and ten univalents. Not flattened.

FIGURE 5. Normal cell in a 41-chromosome plant. Twenty bivalents and one univalent.

FIGURE 6. Aberrant cell in a 41-chromosome plant. Six bivalents and eleven univalents. Not flattened.

FIGURE 7. Normal cell in a 42-chromosome plant. Twenty-one bivalents.

FIGURES 8-11. Aberrant cells in 42-chromosome plants.

FIGURE 8. Three bivalents and nine univalents.

FIGURE 9. Five bivalents and ten univalents.

FIGURES 10 and 11. Contiguous cells with a total of 84 chromosomes.

FIGURE 10. Twenty-nine bivalents and three univalents.

FIGURE 11. Ten bivalents and three univalents.



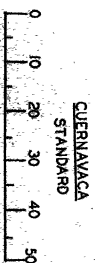
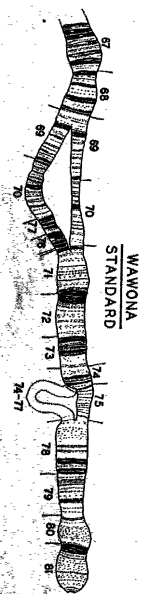
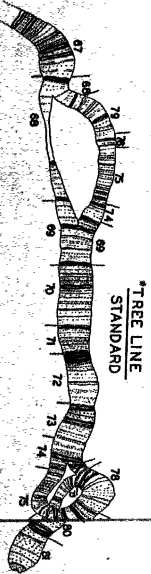
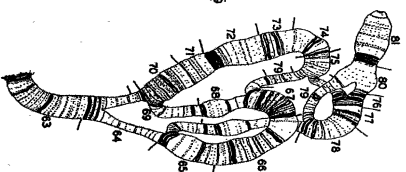
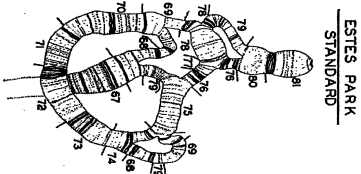
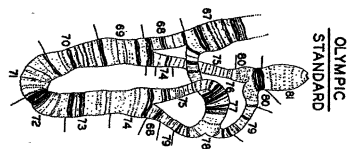
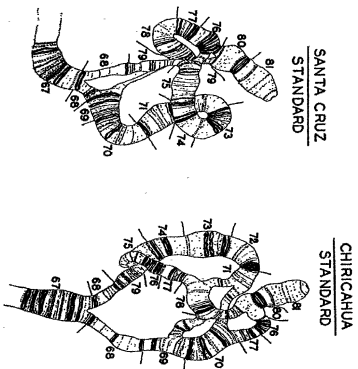
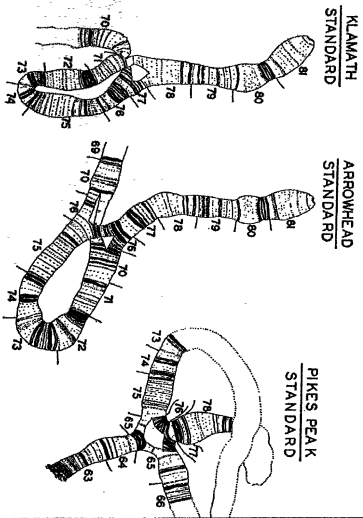
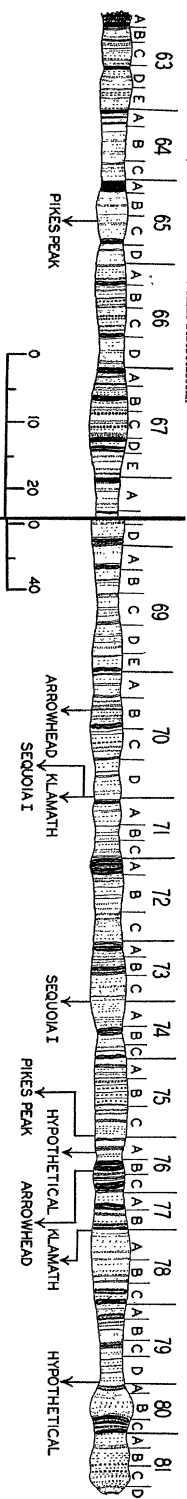






TABLE 2

*The occurrence, and range in chromosome number, of the aberrant pollen mother-cells.*

| MATERIAL                         | ACCESSION<br>NUMBER | CHROMOSOME<br>NUMBER AND<br>ARRANGEMENT<br>CHARACTERISTIC<br>OF PLANT* | NUMBER OF<br>ABERRANT/<br>NORMAL<br>PMC | CHROMOSOME<br>NUMBERS AND<br>ARRANGEMENT IN<br>ABERRANT PMC |
|----------------------------------|---------------------|--|---|---|
| Marquis×Iumillo F <sub>7</sub>   | 36-225.8            | 42 (2II)   | 3/21                                    | 20 (5II)  |
| Iumillo×Hope F <sub>7</sub>      | 36-233.1            | 41 (20II+1I)   | 15/27                                   | 20-30 (6-10II)  |
|                                  | .7                  | 40 (19II+2I)   | 2/20                                    | 32 (11II)   |
|                                  | -235.9              | 42 (2II)   | 1/20                                    | 23 (6II)  |
|                                  | -236.2              | 40 (19II+2I)   | 1/14                                    | 25 (6II)  |
|                                  | -241.8              | 41 (20II+1I)   | 2/32                                    | 22, 23 (5II, 7II)   |
|                                  | -242.6              | 41 (20II+1I)   | 3/50                                    | 16-28 (5-9II)   |
|                                  | .8                  | 39 (19II+1I)   | 2/26                                    | 20 (6II)  |
|                                  | .9                  | 41 (20II+1I)   | 20/49                                   | 20-30 (4-10II)  |
| Iumillo×Hope F <sub>8</sub>      | 36-246.1            | 41 (20II+1I)   | 1/26                                    | 29 (10II)   |
|                                  | .3                  | 41 (20II+1I)   | 3/20                                    | 26 (6II)  |
| Iumillo×R.L. 729 F <sub>8</sub>  | 36-249.9            | 42 (2II)   | 1/30                                    | 34 (6II)  |
|                                  | -252.3              | 42 (2II)   | 1/20                                    | 25 (9II)  |
|                                  | -255.5              | 41 (20II+1I)   | 14/31                                   | 30 (11II)   |
|                                  | -256.7              | 42 (2II)   | 5/20                                    | 15, 27 (3II, 9II)   |
| Marquillo×Iumillo F <sub>8</sub> | 36-271.11           | 42 (2II)   | 2/23                                    | 23, 61 (10II, 29II)   |

\* II signifies bivalent, I signifies univalent.

ties found in his material. KATTERMAN found pollen mother-cells with the following irregularities: in the first metaphase division, an excess of chromatin in addition to the normal set of chromosomes; in early diakinesis, secondary nuclei in addition to the main nucleus; and in zygotene and pachytene, cytomixis, that is, the passage of nuclear material from one pollen mother-cell to another.

TABLE 3

*Chromosome number and arrangement in aberrant pollen mother-cells of plant 36-233.1 (2n=41).*

| CHROMOSOME NUMBER | ARRANGEMENT | FREQUENCY |
|-------------------|-------------|-----------|
| 20                | 6II+8I      | 1         |
| 21                | 6II+9I      | 1         |
| 21                | 7II+7I      | 3         |
| 22                | 6II+10I     | 1         |
| 23                | 6II+11I     | 5         |
| 23                | 7II+9I      | 1         |
| 25                | 7II+11I     | 2         |
| 30                | 10II+10I    | 1         |

The writer (1936) recorded the occurrence of two "haploid" pollen mother-cells in a 42-chromosome derivative of the intra-*vulgare* wheat cross H-44 × Reward. Perhaps of some significance is the origin, in similar material, of a 35-chromosome (14 bivalents and 7 univalents) plant from a selfed 42-chromosome plant.

YASUI (1937) found aberrant pollen mother-cells in an artificially produced *Papaver* hybrid. She observed normal, giant and small pollen mother-cells and attributed the phenomena to hybridity or to aneuploidy. Some of the small pollen mother-cells aborted, and she suggested that this was due to the loss of genes which govern the meiotic process.

TABLE 4

*Chromosome number and arrangement in aberrant pollen mother-cells of plant 36-242.9 (2n=41).*

| CHROMOSOME NUMBER | ARRANGEMENT      | FREQUENCY |
|-------------------|------------------|-----------|
| 20                | 5II + 10I        | 1         |
| 20                | 6II + 8I         | 2         |
| 22                | 4II + 14I        | 1         |
| 26                | 1III + 6II + 11I | 1         |
| 27                | 9II + 9I         | 5         |
| 28                | 7II + 14I        | 3         |
| 28                | 8II + 12I        | 3         |
| 28                | 9II + 10I        | 1         |
| 29                | 7II + 15I        | 1         |
| 29                | 9II + 11I        | 1         |
| 30                | 10II + 10I       | 1         |

In the present material no fragments or extra chromatin were found in any of the aberrant or normal pollen mother-cells. If cytomixis is the cause of their origin it must have occurred at least several cell generations preceding meiosis. Only one anther in a flower has been found with aberrant pollen mother-cells. This limits their origin, in this material, to a state late in ontogeny.

The two contiguous aberrant pollen mother-cells (Plate I, figures 10 and 11) in plant 36-271.11 (2n=42) contained a total of 84 chromosomes: 29 bivalents and 3 univalents, and 10 bivalents and 3 univalents, respectively. It appears that failure of cytokinesis in a spermatogenous cell resulted in one with 84 chromosomes. An atypical premeiotic mitosis then gave rise to the two pollen mother-cells, one with 61 and the other with 23 chromosomes.

Each of the 14 aberrant pollen mother-cells in plant 36-255.5 (2n=41) had 30 chromosomes (11 bivalents and 8 univalents). It is logical to assume that an atypical division occurred at least four cell generations before meiosis and that the sister cell with 11 chromosomes was eliminated.

(The lowest aberrant chromosome number observed in this material was 15 (Plate I, figure 8). Compare also the abortion of the small pollen mother-cells reported by YASUI (1937).)

On the other hand, the aberrant pollen mother-cells in some plants exhibited a wide range with respect to chromosome number (tables 3 and 4). Presumably these owe their origin to one atypical mitosis in each plant, followed by irregularities in succeeding cell generations, or to atypical mitoses of several normal cells. The aberrant pollen mother-cells are usually characterized by relatively large numbers of unpaired chromosomes (tables 2, 3 and 4; Plate I, figures 2, 4, 6, 8 and 9).

Since more than four percent of the 42-chromosome plants contained aberrant pollen mother-cells, their origin in others can not be attributed to an aneuploid condition of the plants. An upset in mitosis seems to be the cause of the origin of these hypo- and hyperploid pollen mother-cells. In hybrid material such as this, atypical mitoses are probably due to mutually incompatible genes contributed by the different parents to some of their progeny, or to incompatible cytoplasm-gene combinations. The fact that the Iumillo  $\times$  Hope derivatives (which have three species in their parentage) contributed over one-half of the aberrant plants, though including less than one-third of the material examined, emphasizes the rôle of hybridity in producing such incompatibilities. The specificity of the reactions involved is, at the same time, indicated by the complete regularity, so far as examined, of the remaining 320 hybrid plants, of which 184 were unbalanced, aneuploid types.

#### ACKNOWLEDGMENTS

The writer wishes to express his thanks to Dr. R. F. PETERSON who supplied the seed which made this study possible, and to Dr. C. L. HUSKINS, Department of Genetics, McGill University, for criticisms in the preparation of the manuscript.

#### SUMMARY

1. Pollen mother-cells with less (and one with more) than  $2n$  chromosomes are recorded in 16 out of 336 plants derived from pentaploid wheat hybrids.
2. The chromosome number in the aberrant pollen mother-cells ranged from 15 to 61; the number of bivalent chromosomes varied from 3 to 29.
3. Their occurrence in only one anther of a plant limits their origin to a stage late in ontogeny.

#### APPENDIX

Since the above paper was completed, the writer has observed two hypoploid pollen mother-cells with 18 chromosomes (5 bivalents and 8

univalents) in a dwarf, sterile, 40-chromosome (20 bivalents) winter wheat plant. The plant arose in an off-type line of the variety Dawson's Golden Chaff (*T. vulgare*). The conclusion reached earlier is emphasized, namely, that the aberrant pollen mother-cells reported in the above paper are not due to hybridity *per se*, but to incompatible gene combinations in certain hybrid derivatives.

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# STUDIES ON SPOTTING PATTERNS. IV. PATTERN VARIATION AND ITS DEVELOPMENTAL SIGNIFICANCE

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Received June 5, 1938

## INTRODUCTION

PERHAPS the most striking feature of the spotting patterns found in individuals or strains of many mammalian species is their great variety. They seem all to have the same immediate basis, failure of pigmentogenesis in the hair follicles of some portion of the skin. But the site of the failure is almost unique to each spotted individual.

Considerable variability persists even in strains where the chance of genetic segregation has been minimized by long inbreeding (DUNN 1920). For example, one strain reported in the first paper of this series (DUNN and CHARLES 1937) included individuals with few or no white hairs dorsally, others with unpigmented fur on as much as 25 percent of the dorsum, and a majority of intermediate grade. Among mice with about the same amount of white some had the unpigmented areas only on the left side, some only on the right and some on both.

That there is, however, a certain order in the intra-strain variation of spotting pattern has long been recognized (ALLEN 1914). Thus, in the strain cited above, no individual bore white hairs dorsally elsewhere than on the lumbar region and the distal portions of the legs and tail. Furthermore within the general lumbar area the central portion seems to be more frequently unpigmented than portions either more anterior or more posterior. Similarly there were more mice with white fur only on the feet and tail tip than with white fur extending more proximally on the legs and tail. Thus different body regions are much less sharply differentiated in their non-genetic variation than they are in the single spotted individual, as WRIGHT (1920) has pointed out. Each small skin area has a characteristic potentiality for developing pigment measurable by the proportion of animals within a strain bearing pigmented fur on the area; and the potentiality seems to vary systematically from area to area over the body surface, forming some sort of a gradient field which depends presumably on

<sup>1</sup> Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy of Columbia University. The writer is grateful to the Trustees and Council of Columbia University for a University Fellowship in Zoology (1933-4) during tenure of which part of the present work was done; and to Professor L. C. Dunn for many kindnesses during the course of the investigation.



=L66; 20% = LK; 35% = L190; 100% = L19. The hybrids will be denoted by the product of the parent averages; for example (20% $\times$ 100%) = LK $\times$ L19.

The 4% and 35% lines each carry the piebald gene "s" of the older literature. The 100% line has this gene and in addition at least three other mutant alleles tending to produce white spotting and acting cumulatively with s. The 20% strain has the wild type allele of s but otherwise carries most or all of the mutant alleles of the 100% line. The main ones of this latter group may be designated by *k*, *e*, *f*; it is probable that there are a few other mutant genes with minor effects in the 20% and 100% stocks. The tentative genotypes of the four strains are: 4% *ssKKKEEFF*; 20%, *SSkkeeff*; 35%, *ssKKKEeff*; 100%, *sskkeeff*, wild type being *SSKKKEEFF*. For the present purposes the precise number of genes differentiating the strains is less important than the clear fact that the pattern differences among strains are genetically determined (DUNN and CHARLES 1937).

### Method

Camera lucida tracings were made of the spotting patterns of the following mice, each at  $10 \pm$  days of age: 27 *ssKkEeff* (35% $\times$ 100%); 31 *ssKkEeFf* (4% $\times$ 100%); 21 *ssKKKEeff* (35%); 48 *Sskkeeff* (20% $\times$ 100%); 94 *SSkkeeff* (20%); and 56 *ssKKKEEFF* (4%). At the age when tracings were made the hairs are still quite short so that the superficial pattern corresponds closely with that at the surface of the skin.

Each mouse was etherized and so arranged beneath the camera lucida that its projected outline coincided as closely as possible with a standard diagram drawn on quadrille paper with tenth-inch rulings; the projected boundaries between colored and white areas were then traced on the diagram. (It was not always possible to make the two outlines coincide completely; in these cases the pattern boundaries were not traced exactly but were shortened or lengthened proportionately.) There were thus obtained a number of identical-area pattern records, identically subdivided.

Two representative tracings from each of the six genotypic groups are shown in figure 1. (In this figure the original quadrille rulings have not been reproduced and the areas bearing colored fur have been blacked in.)

### Data

From the pattern tracings was obtained the "frequency of pigmentation" at each of 508 skin points for each of the six genotypic groups. This was done simply by counting what proportion of the tracings in a group showed pigmented fur at the center of the first square, what proportion at the center of the second square, and so on for each of the 508 squares into which the quadrille rulings divide the standard diagram.

The frequencies so obtained are represented in figures 2-4 where the height of each square pillar is the proportion of mice of the given genotype bearing pigmented fur at the center of the skin region beneath the pillar.

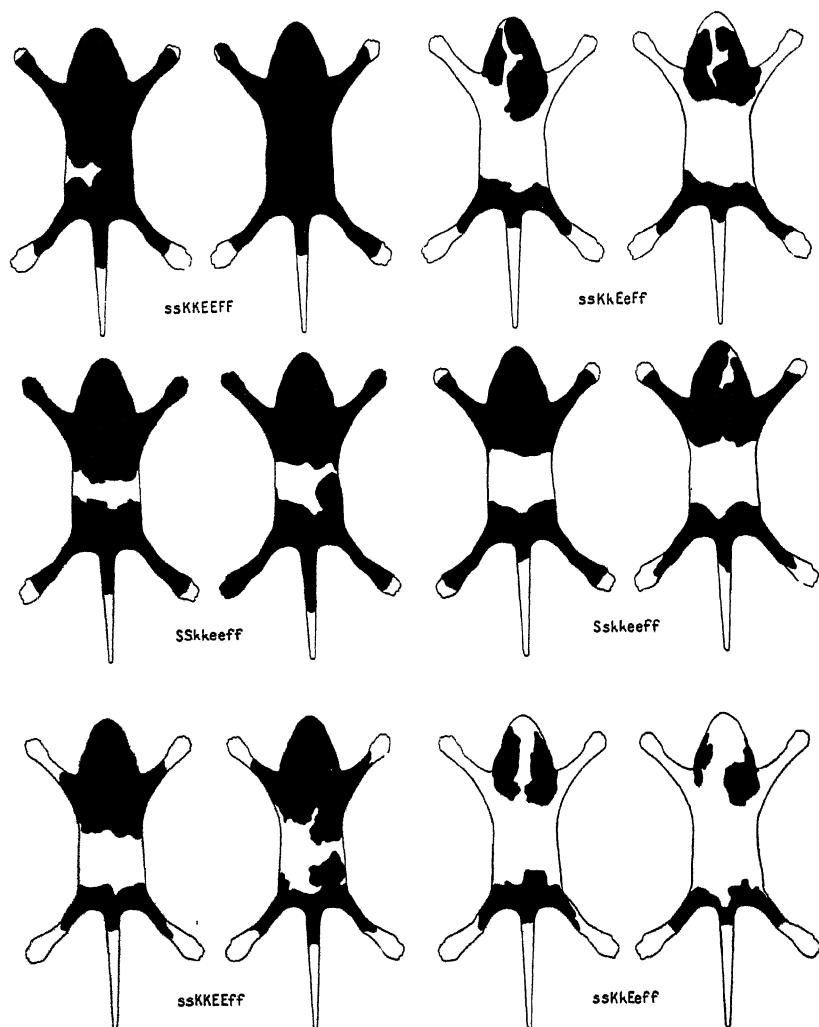


FIGURE 1.—Representative dorsal patterns of six genetic types of spotted mice.

Each figure is a gradient field measuring the regionally-variable potentiality for forming pigment in the presence of a particular gene combination.

The frequencies in figures 2-4 can be identified with "points" of skin only with certain qualifications arising as follows. In tracing the spotting



patterns, as has been indicated, it was not always possible to arrange the mouse to coincide exactly with the outlines of the diagram. In such cases

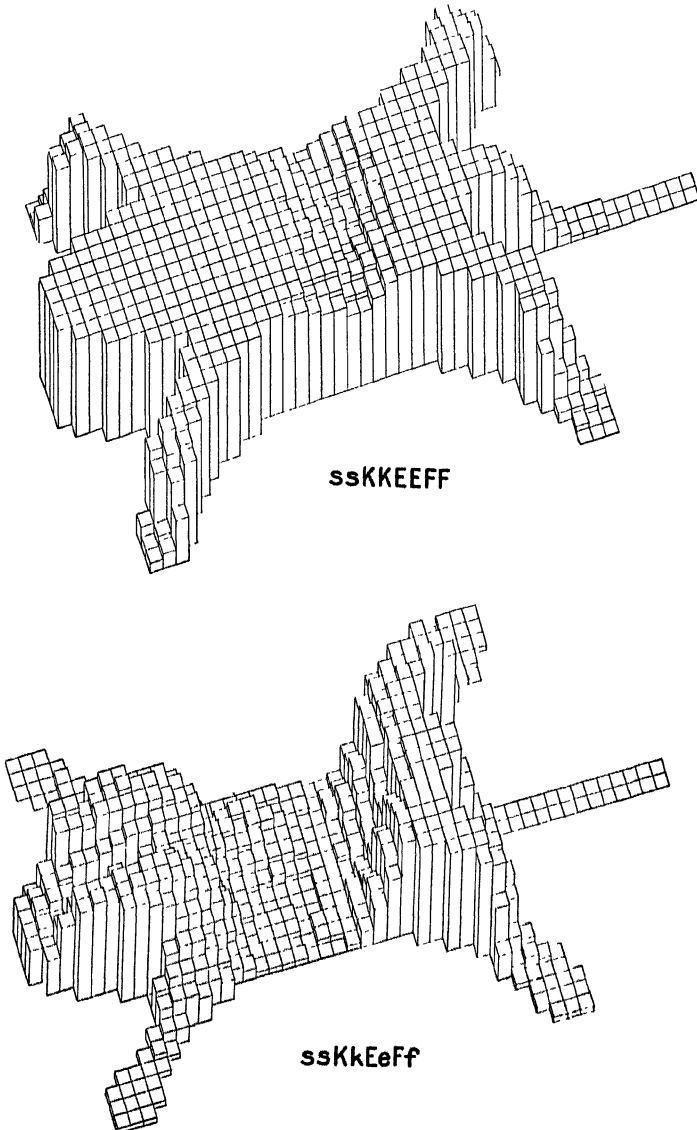


FIGURE 2.—Pigmentation frequencies at 508 skin points in *ssKKEEFF* and *ssKkEeFf* spotted mice.

it was necessary to enlarge or reduce portions of the projected pattern in the tracing process. In addition, slight changes in position of the eye during tracing tended to produce irregular shifts in position of the projected

pattern. In consequence even repeated tracings of the same mouse would be expected to show the same point of skin represented at somewhat different positions on the diagram. Conversely, a constant point of the diagram corresponds to somewhat different points on the different skins traced. The range of this uncertainty can scarcely have been less than 1 percent of the distance from nose tip to tail base, nor much more than 3 percent. Consequently the frequency of pigmented fur represented at a given point in a set of tracings is a complex average of the actual frequencies within an area of diameter equal to 1-3 percent of the body length. For convenience the frequencies will be referred to subsequently as though they described events at actual points of skin, but the limitation may be borne in mind.

The six gradient fields of figures 2-4 are represented as contour maps in figure 5. Each of these maps has been made by plotting on a standard diagram the series of points where the pigmentation frequency of one genotype is 10, 30, 50, 70 and 90 percent; in most cases the points were located by interpolation. The points were then connected by smooth lines, but they give a fair approximation to the contours of the three-dimensional figures.

It will be noticed that several of the maps have contour lines at very nearly the same location. The 30 and 70 percent lines on the head of *ssKkEeff* correspond very closely to the 50 and 90 percent lines, respectively, of *ssKkEeFf*. The 50 percent line on the anterior dorsum of *Sskkeeff* corresponds to the 90 percent line of *SSkkeeff*. Also in general the contour lines on each part of the body run in nearly the same direction for all genotypes, regardless of the actual values. Altogether it seems as though isopotential, that is similarly reacting, strips of tissue were being dealt with: whatever the pigmentation frequency of a particular point may be in the presence of a particular gene combination, all other points on the strip have very nearly the same value.

Two other general relations are readily seen by comparisons among the data of figures 2-4, as follows.

*Relative height of frequency field in homozygote and heterozygote.* Three such comparisons can be made: *ssKKEEFF* with *ssKkEeFf* (figure 2); *SSkkeeff* with *Sskkeeff* (figure 3) and *ssKKEEff* with *ssKkEeff* (figure 4). In each case the homozygote can be seen to have a higher pigmentation potentiality than the heterozygote at every point (except where both are 0 or 100 percent) as might be expected. That is, substitution of the wild type genes for the mutant alleles simply increases the pigmentation frequency at each point of the skin (except where the frequency is already 100 percent in the heterozygote). As to the magnitude of increment, the most obvious possibility is that it should be everywhere the same. That

such is not the case is shown by table 1: the increment varies with the pigmentation frequency in the heterozygote, and perhaps with the general

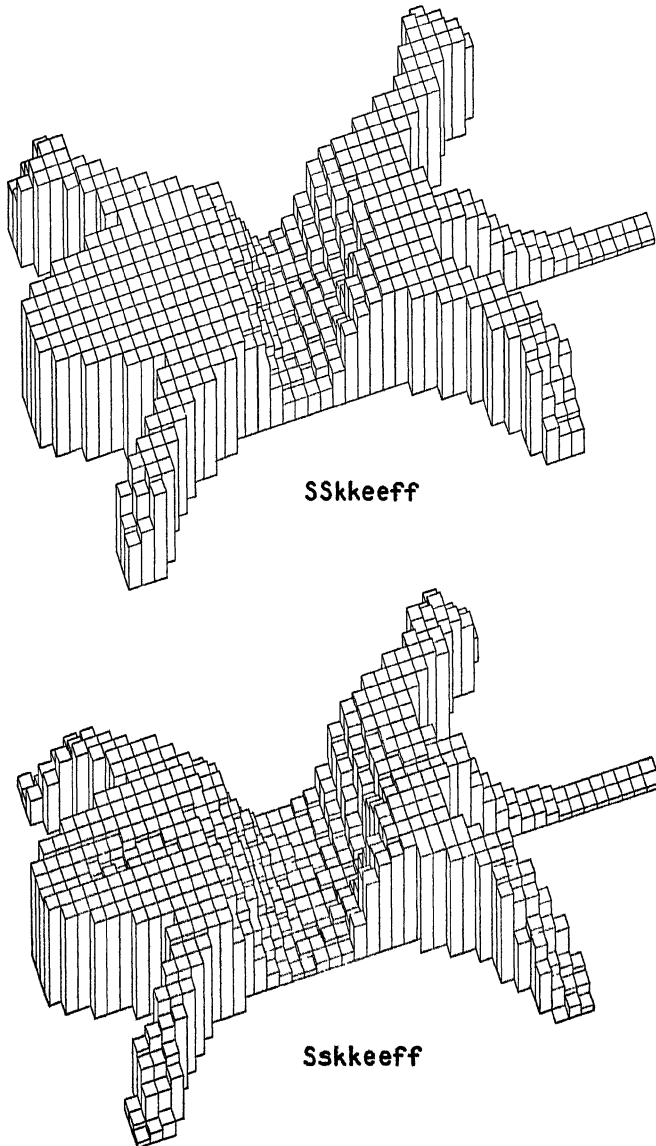


FIGURE 3.—Pigmentation frequencies at 508 skin points in *Sskkeeff* and *Sskkeeff* spotted mice.

region of the body. However, as will be seen in the analytical section, an approach to constancy of increment from heterozygote to homozygote is obtained if the pigmentation frequencies are transformed in a simple way.

*Action of different loci on the frequency field.* The simplest expectation here is that the six gradient fields should have the same general form and fall into a simple series with respect to height, their order being the same at every point of the skin. Such a situation might be expected if the *s*, *k*,

TABLE I

*Pigmentation frequency, in percent, of corresponding skin points in heterozygous and homozygous spotted mice; the increments from heterozygote to homozygote are shown in brackets.*

| <i>Sskkeeff</i> (56 MICE) |              | AVERAGE FREQUENCY* IN <i>SSkkeeff</i> (94 MICE) |                     |                    |          |      |                 |
|---------------------------|--------------|---|---------------------|--------------------|----------|------|-----------------|
| RANGE                     | AVER-<br>AGE | HIND LEGS<br>AND TAIL                           | POSTERIOR<br>DORSUM | ANTERIOR<br>DORSUM | FORELEGS | HEAD | TOTAL<br>POINTS |
| 1-9                       | 5            | 18 (13)   | 34 (29)             | 25 (20)            | 47 (42)  | —    | 48              |
| 10-18                     | 14           | 30 (16)   | 42 (28)             | 38 (24)            | 55 (41)  | —    | 42              |
| 19-26                     | 22           | 49 (27)   | 58 (36)             | 61 (39)            | 79 (57)  | —    | 19              |
| 27-34                     | 30           | 70 (40)   | 74 (44)             | 67 (37)            | 92 (62)  | —    | 12              |
| 35-43                     | 39           | 86 (47)   | 84 (45)             | 84 (45)            | 79 (40)  | —    | 10              |
| 44-51                     | 47           | 79 (32)   | —                   | 93 (46)            | 97 (50)  | —    | 8               |
| 52-59                     | 55           | 97 (42)   | 95 (40)             | 96 (41)            | 97 (42)  | —    | 13              |
| 60-68                     | 64           | 95 (31)   | 95 (31)             | 100 (36)           | 97 (33)  | —    | 9               |
| 69-76                     | 72           | 100 (28)  | 95 (23)             | 100 (28)           | 97 (25)  | —    | 17              |
| 77-84                     | 80           | 100 (20)  | 98 (18)             | 100 (20)           | 98 (18)  | —    | 29              |
| 85-93                     | 89           | 100 (11)  | 98 (9)              | 100 (11)           | 100 (11) | —    | 17              |
| 94-99                     | 96           | 98 (2)  | 100 (4)             | 100 (4)            | 100 (4)  | —    | 46              |
| 100                       | —            | 100   | —                   | 100                | 100      | —    | 79              |

| <i>ssKkeeff</i> (27 MICE) |    | AVERAGE FREQUENCY IN <i>ssKKEEff</i> (21 MICE) |          |          |         |          |     |
|---------------------------|----|--|----------|----------|---------|----------|-----|
| 0                         | —  | 9  | 4        | 27       | 41      | —        | 137 |
| 1-9                       | 5  | 9 (4)  | 6 (1)    | 48 (43)  | 42 (37) | —        | 98  |
| 10-17                     | 13 | 33 (20)  | 32 (19)  | 73 (60)  | 64 (51) | 96 (83)  | 34  |
| 18-24                     | 21 | 36 (15)  | 40 (19)  | 76 (55)  | 74 (53) | 94 (73)  | 23  |
| 25-32                     | 29 | —  | 59 (30)  | 85 (56)  | —       | 100 (71) | 12  |
| 33-39                     | 36 | —  | 83 (47)  | 89 (53)  | —       | 100 (64) | 15  |
| 40-47                     | 44 | 55 (11)  | 78 (34)  | 93 (49)  | —       | 99 (55)  | 28  |
| 48-54                     | 51 | —  | 77 (26)  | 93 (42)  | —       | 100 (49) | 14  |
| 55-62                     | 58 | 51 (-7)  | 95 (37)  | 95 (37)  | —       | 100 (42) | 17  |
| 63-69                     | 66 | 71 (5)   | —        | 100 (34) | —       | 100 (34) | 10  |
| 70-77                     | 73 | 82 (9)   | 92 (19)  | —        | —       | 100 (27) | 15  |
| 78-84                     | 81 | 96 (15)  | 94 (13)  | —        | —       | 100 (19) | 23  |
| 85-91                     | 88 | 97 (9)   | 100 (12) | —        | —       | 100 (12) | 20  |
| 92-99                     | 95 | 98 (3)   | 100 (5)  | —        | —       | 100 (5)  | 18  |
| 100                       | —  | —  | 100      | 100      | —       | 100      | 20  |

*e* and *f* alleles all had the same sort of primary effect, but different degrees of activity, as WRIGHT (1920) has assumed to be the case for a group of spotting genes in the guinea pig. The expectation seems to be fulfilled in the case of *ssKKEEFF* (4%), *ssKKEEff* (35%), *ssKkeEef* (4% × 100%) and *ssKkeEff* (35% × 100%). Six comparisons can be made among these genotypes, taking two at a time: *ssKKEEFF* and *ssKKEEff* have already

been seen to be consistently higher than *ssKkEeFf* and *ssKkEeff*, respectively; direct comparison of figures 2 and 4 shows that *ssKKEEFF* is

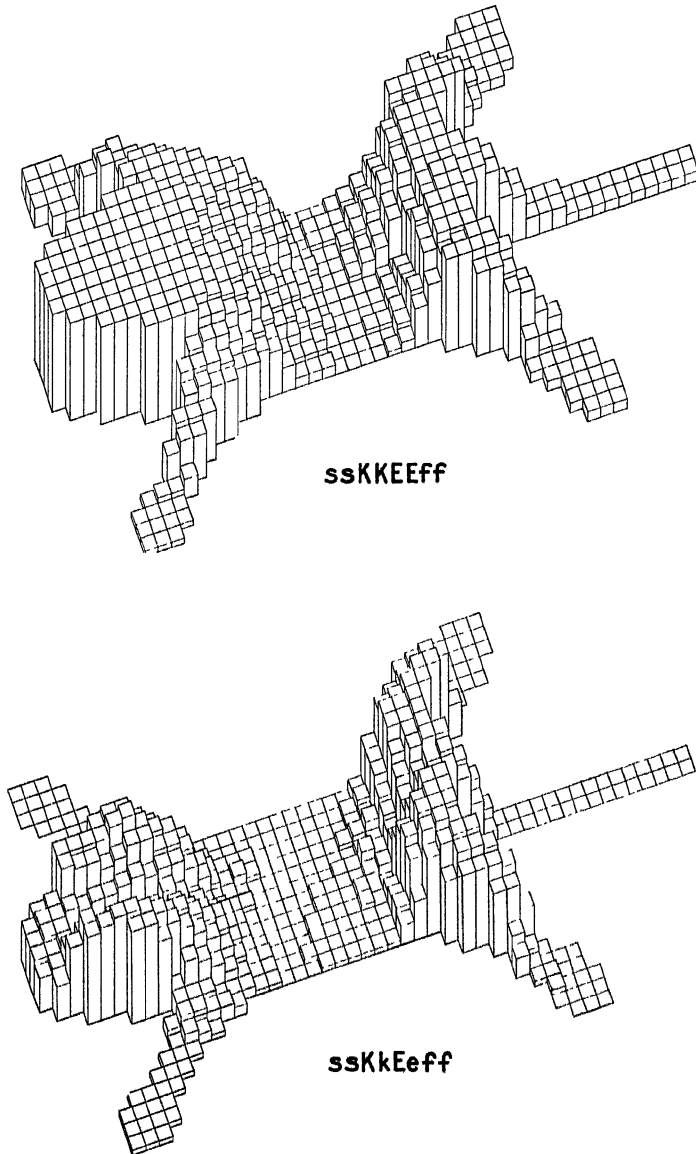


FIGURE 4.—Pigmentation frequencies at 508 skin points in *ssKKEEff* and *ssKkEeff* spotted mice.

higher than *ssKkEeff*, *ssKKEEff* higher than *ssKkEeFf*. Two comparisons remain to be made: *ssKKEEFF* (4%) with *ssKKEEff* (35%) and *ssKkEeFf* (4%  $\times$  100%) with *ssKkEeff* (35%  $\times$  100%); these are shown

in table 2. The *ssKKEEFF* field is higher than that of *ssKKEEff*, or equal to it at 100 percent, except at 42 of the 508 points. Nearly all of these points are on the extreme distal portions of the hind legs and tail where the data are least reliable since only few animals of either genotype bear pigmented fur there.

The same sort of relation is found between the frequency fields of *ssKkEeFf* and *ssKkEeff*. The former is equal to, or greater than, the latter at 431 of the 495 points recorded in table 2. Of the remaining 64 points, 48 bore pigmented fur in none, or only one, of the 31 *ssKkEeFf* mice and in one, or two, of the 27 *ssKkEeff* mice; the other 16 points had very nearly

TABLE 2

*Relative frequency of pigmentation at corresponding skin points of different spotting genotypes.*

| RELATIVE FREQUENCY                                | NUMBER OF POINTS      |                          |                         |               |      |                 |
|---|-----------------------|--------------------------|-------------------------|---------------|------|-----------------|
|   | HIND LEGS<br>AND TAIL | POS-<br>TERIOR<br>DORSUM | AN-<br>TERIOR<br>DORSUM | FORE-<br>LEGS | HEAD | TOTAL<br>POINTS |
| <i>ssKKEEFF</i> > <i>ssKKEEff</i>                 | 55                    | 86                       | 117                     | 59            | 6    | 323             |
| <i>ssKKEEFF</i> < <i>ssKKEEff</i>                 | 39                    | 0                        | 0                       | 3             | 0    | 42              |
| <i>ssKKEEFF</i> = <i>ssKKEEff</i><br>= 100%       | 24                    | 28                       | 3                       | 0             | 88   | 147             |
| <i>ssKkEeFf</i> > <i>ssKkEeff</i>                 | 83                    | 80                       | 95                      | 34            | 80   | 372             |
| <i>ssKkEeFf</i> < <i>ssKkEeff</i>                 | 2                     | 20                       | 11                      | 17            | 14   | 64              |
| <i>ssKkEeFf</i> = <i>ssKkEeff</i><br>= 0% or 100% | 30                    | 11                       | 7                       | 11            | 0    | 59              |
| <i>ssKKEEff</i> > <i>Sskkeeff</i>                 | 22                    | 8                        | 44                      | 2             | 29   | 105             |
| <i>ssKKEEff</i> < <i>Sskkeeff</i>                 | 68                    | 80                       | 73                      | 50            | 6    | 277             |
| <i>ssKKEEff</i> = <i>Sskkeeff</i><br>= 100%       | 24                    | 26                       | 3                       | 0             | 59   | 112             |

equal pigmentation frequencies. Since the differences at these discordant points are so small, they may perhaps be only a consequence of sampling error.

Thus it seems probable that the frequency fields of *ssKKEEFF*, *ssKKEEff*, *ssKkEeFf* and *ssKkEeff* fall into a consistently diminishing series in the order indicated and that the *KE* and *F* wild type genes may thus have the same sort of primary or intermediate action.

There seems however to be a different relation between the genotypes containing *KE* without *S* and those containing *S* without *KE*. The most extensive comparison available is between *ssKKEEff* (35%) and *Sskkeeff* (20% × 100%), in table 2. The significant regions here are the head, where *Sskkeeff* often shows a white blaze although *ssKKEEff* never does,

and the posterior dorsum, where *Sskkeeff* has consistently higher pigmentation frequencies. A similar reversal in relative height of fields can be seen by comparing figures 2 and 3; over the general lumbar area, *ssKKEEFF* has much higher pigmentation frequencies than *SSskkeeff*; but on the distal halves of the legs *SSskkeeff* is higher. It seems very unlikely, from the sizes of the two samples and from the range of frequencies over which the reversal is observed, that the reversal is only a matter of sampling error.

*SSskkeeff* (20%) and *Sskkeeff* (20%  $\times$  100%) thus do not fit into a consistent series with the other four genotypes, but have a pigmentation frequency field of fundamentally different shape. It seems probable accordingly that *S* has a different primary or intermediate action than *K*, *E* and *F*.

*Correlations in pattern variation.* A fourth general phenomenon of the variation in spotting pattern is the interrelation in pigmentation response (that is, forming or not forming pigment) among large groups of skin points. This has been previously studied by ALLEN (1914) in genetically heterogeneous groups from various mammalian species and by ILJIN (1928) in guinea pigs. The immediately observable fact is that the skin of any spotted animal (except in the case of dominant spotting) consists of a small number of large pigmented or unpigmented regions. Furthermore there is a limited variation in the arrangement of the two kinds of regions, within any homogeneous stock. In the lighter genotypes (*ssKkEeFf*, *ssKkEeff*) there are typically three patches of colored fur dorsally: one covering both sides of the rump and extending somewhat onto the legs and tail; one centering on each ear and extending towards the eyes and scapula. The two ear patches may be confluent; there may be an "island" of colored fur somewhere between the ear and rump patches. In the darker genotypes (*ssKKEEFF*, *SSskkeeff*) there are rarely more than six regions of white fur, dorsally; one on each foot, one on the tail tip and one on the lumbar area. The foot and tail patches may extend for various distances centrally; there are occasionally two lumbar patches; in *SSskkeeff* there is sometimes a white blaze on the forehead. In all groups no part of the leg or tail is pigmented unless there are also pigmented hairs on all more proximal parts of the appendage at least as far as the point indicated by the intersection of the dotted lines from leg and tail in the *ssKKEEFF* contour map in figure 5. Conversely if the knee, or tail base, bears *white* hairs, all more distal parts of the appendage do also. No part of the foreleg is pigmented unless there are pigmented hairs as far centrally as the base of the ear (intersection of the dotted lines on arm and body proper in figure 5). The anterior tip of the head is never pigmented unless there is colored fur at least as far posteriorly as the rear of the eye.

Although leg and tail pigmentation seem thus to depend on the formation of pigment more centrally they do not depend on each other. That is (in *SSkkeeFF*, *SskkeeFF* and *ssKKEEFF*) the proportion of animals with pigment at any one point on the leg and any one point on the tail has not

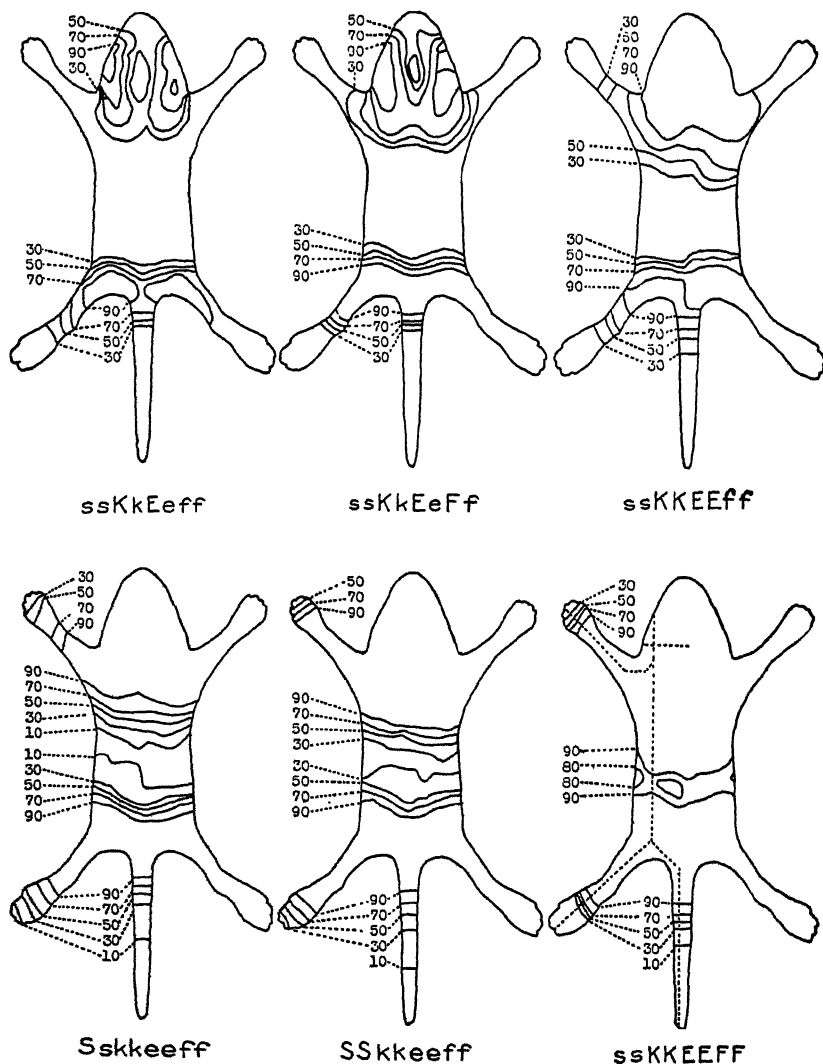


FIGURE 5.—Contours of the pigmentation frequency surfaces in figures 2-4.

been found to be significantly different from the chance expectation, that is, from the product of the pigmentation frequencies of the two points. Similarly no relation has been found between pigmentation of points on the tail and foreleg, on hind and fore leg or on opposite sides of the body.



A somewhat different situation is found in the central two-thirds of the body proper, that is, the central two-thirds of the longitudinal dotted line on the *ssKKEEFF* contour map in figure 5. The coefficients of association in pigmentation at pairs among ten points along this axis are shown in table 3, for *SSkkeeFF* which is the only group of tracings large enough to give fairly reliable results. There is a consistent association in presence or absence of pigment between any pair of adjacent points and

TABLE 3

*Association in presence or absence of pigment at ten equidistant points extending from shoulder to rump (along the dotted line in figure 5) in SSkkeeFF spotted mice.  $N_i$  is the number of animals with pigment at each of two points;  $N_i$  with pigment at the more anterior point only;  $N_1$ , at the most posterior point only;  $N_0$ , at neither;  $c$  is the coefficient of association,  $(N_{ii} \cdot N_0 - N_i \cdot N_j) / (N_{ij} \cdot N_0 + N_i \cdot N_j)$ .*

| POINTS | $N_{ij}$ | $N_i$ | $N_j$ | $N_0$ | $c$   | POINTS | $N_{ij}$ | $N_i$ | $N_j$ | $N_0$ | $c$   |
|--------|----------|-------|-------|-------|-------|--------|----------|-------|-------|-------|-------|
| 1.2    | 52       | 30    | 0     | 12    | 1     | 3.9    | 17       | 6     | 59    | 12    | -0.27 |
| 1.3    | 23       | 59    | 0     | 12    | 1     | 3.10   | 21       | 2     | 69    | 2     | -0.53 |
| 1.4    | 21       | 61    | 2     | 10    | 0.27  | 4.5    | 19       | 4     | 5     | 66    | 0.97  |
| 1.5    | 23       | 59    | 1     | 11    | 0.62  | 4.6    | 17       | 6     | 14    | 57    | 0.84  |
| 1.6    | 28       | 54    | 3     | 9     | 0.22  | 4.7    | 16       | 7     | 22    | 49    | 0.67  |
| 1.7    | 31       | 51    | 7     | 5     | -0.39 | 4.8    | 17       | 6     | 33    | 38    | 0.53  |
| 1.8    | 41       | 41    | 9     | 3     | -0.50 | 4.9    | 17       | 6     | 59    | 12    | -0.27 |
| 1.9    | 65       | 17    | 11    | 1     | -0.48 | 4.10   | 20       | 3     | 70    | 1     | -0.83 |
| 1.10   | 78       | 4     | 12    | 0     | -1    | 5.6    | 21       | 3     | 10    | 60    | 0.95  |
| 2.3    | 21       | 31    | 2     | 40    | 0.86  | 5.7    | 19       | 5     | 19    | 51    | 0.82  |
| 2.4    | 14       | 38    | 9     | 33    | 0.15  | 5.8    | 20       | 4     | 30    | 40    | 0.74  |
| 2.5    | 14       | 38    | 10    | 32    | 0.08  | 5.9    | 20       | 4     | 56    | 14    | 0.11  |
| 2.6    | 17       | 35    | 14    | 28    | -0.01 | 5.10   | 21       | 3     | 69    | 1     | -0.82 |
| 2.7    | 17       | 35    | 21    | 21    | -0.35 | 6.7    | 28       | 3     | 10    | 53    | 0.96  |
| 2.8    | 24       | 38    | 26    | 16    | -0.31 | 6.8    | 28       | 3     | 22    | 41    | 0.94  |
| 2.9    | 40       | 12    | 36    | 6     | -0.29 | 6.9    | 27       | 4     | 49    | 14    | 0.32  |
| 2.10   | 50       | 2     | 40    | 2     | 0.11  | 6.10   | 27       | 4     | 63    | 0     | -1    |
| 3.4    | 16       | 7     | 7     | 64    | 0.91  | 7.8    | 32       | 6     | 18    | 38    | 0.84  |
| 3.5    | 12       | 11    | 12    | 59    | 0.69  | 7.9    | 36       | 2     | 40    | 16    | 0.74  |
| 3.6    | 13       | 10    | 18    | 53    | 0.59  | 7.10   | 36       | 2     | 54    | 2     | -0.20 |
| 3.7    | 12       | 11    | 26    | 45    | 0.31  | 8.9    | 43       | 3     | 29    | 15    | 0.78  |
| 3.8    | 15       | 8     | 35    | 36    | 0.31  | 9.10   | 74       | 2     | 16    | 2     | 0.64  |

a decreasing association between points successively further apart. From each point the association falls to zero and then assumes negative values.

The meaning of these negative values is not clear, but they must arise in part from the tracing method. The inaccuracies of alignment of the projected outline of a mouse against a standard diagram are such that, if a given mouse were traced repeatedly, the boundaries of a given white spot would not lie identically on the tracing. In general where the anterior boundary of the spot has been traced too far forward, the posterior boundary will also be too anterior; where the front boundary has been traced

further back, the rear boundary will also be relatively posterior. In repeated tracings of the same mouse there should thus be an association coefficient of  $-1$  between presence of pigment at one point and another separated from it by the length of the spot traced. In tracings of different mice there will be a tendency to similar negative association between any two points which may lie immediately fore and aft of a white spot. Because of this error it is difficult to attach any meaning to the negative coefficients in table 3; the positive values indicate a real association.

In general, the formation of pigment on either the posterior or anterior dorsum shows a decreasing association with pigment on points successively nearer the opposite end of the body. Formation of pigment in the mid-dorsum is decreasingly associated with the pigmentation response of points successively further away either anteriorly or posteriorly. It seems probable that a similar relation holds for all six genotypes.

#### ANALYTICAL

*Pigmentation frequencies.* WRIGHT (1920, 1936) has used an "inverse probability transformation" to convert the proportion of white fur in the skin of a spotted guinea pig into a quantity which is equivalent to an average "concentration" of some material throughout the skin, relative to a "critical concentration" for pigment formation. The same transformation may be used to convert the pigmentation frequency of one skin point within a genotype into an "average concentration" at that point. The basis of the transformation is as follows.

"Although the skin of a piebald guinea pig is divided sharply into areas in which pigment is either produced to the full amount characteristic of the animal, or is wholly absent, it is not to be supposed that the influences, which at some critical period in ontogeny determine whether a region is to be colored or white, are so sharply alternative in themselves. It seems more reasonable to suppose that the sums of favorable and unfavorable influences in different parts of the skin could be arranged in a graded series. Doubtless in certain white regions a slight difference in the conditions would have enabled color to develop, while in others a great change would have been necessary. Similarly with colored areas. Suppose, then, that the skin is divided into a large number of equal areas and that it were possible to determine the true potentiality of each area at the critical period in development. . . . All areas which exceed this a 'critical potential' produce color, while those which fall below, however slightly, remain white." (WRIGHT 1920.)

The "true potentiality" of the cells ancestral to a small area of a *particular skin* at the "critical period in ontogeny" might be thought of, in the simplest possible way, as a concentration  $P$  of some unknown material.

The "critical potential" would then be the smallest concentration  $P_0$  which could bring about the ultimate formation of pigment. Pigmented hairs would be borne on an area only if  $P$  had been equal to, or greater than,  $P_0$ . Since some regions of an individual bear pigmented fur, others white fur, either  $P$  or  $P_0$  or both must vary from region to region of the same skin. Since the same small skin area may have pigmented fur in some individuals and white fur in other individuals of the same genotype, either  $P$  or  $P_0$ , or both, of that area must vary somewhat from animal to animal,  $P$  being the greater in some,  $P_0$  in others. Since the spotting patterns are at least roughly similar among animals of the same genotype, the regional variation in  $P$  or  $P_0$  must have the same general form throughout the group; that is, both  $P$  and  $P_0$  must tend to a modal value for each skin

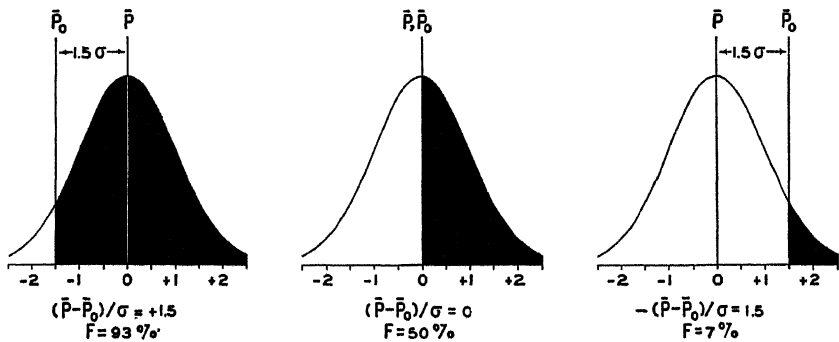


FIGURE 6.—Relation of pigmentation frequency ( $F$ , shaded area) at a skin point to average difference between a variable "concentration"  $P$  at that point and a minimal "concentration"  $P_0$  for pigment formation (modified from Wright 1920).

point among individuals of the same genotype. Let us assume that the frequencies of various values of the difference  $(P - P_0)$  for a given small skin area would form a normal distribution of standard deviation  $\sigma$ ; the average value of the difference may be represented by  $(\bar{P} - \bar{P}_0)$ . The pigmentation frequency  $F$  to be expected for any value of  $(\bar{P} - \bar{P}_0)$ , relative to  $\sigma$ , can be found from a table of areas under the normal curve. That is,  $F$  is the probability function of  $(\bar{P} - \bar{P}_0)/\sigma$ . Three examples are shown in figure 6.

Conversely, the value of  $(\bar{P} - \bar{P}_0)/\sigma$  corresponding to a particular  $F$  can be found from a table of the normal curve areas; that is,  $(\bar{P} - \bar{P}_0)/\sigma$  is the inverse probability function of  $F$ . A few pairs of values are as follows:

|                                |       |       |       |       |       |    |       |       |       |       |       |
|--------------------------------|-------|-------|-------|-------|-------|----|-------|-------|-------|-------|-------|
| $F$ (in %)                     | 1     | 2     | 5     | 10    | 20    | 50 | 80    | 90    | 95    | 98    | 99    |
| $(\bar{P} - \bar{P}_0)/\sigma$ | -2.32 | -2.06 | -1.64 | -1.28 | -0.84 | 0  | +0.84 | +1.28 | +1.64 | +2.06 | +2.32 |

This inverse probability transformation might be applied to each of the values in figures 2-4. No additional information would be gained, though: the relative steepness of various parts of the surfaces would be changed, but the order of their height, and the regional reversal of height between

*Skef* and *sKEF* genotypes, would not be altered. Insofar, however, as  $(\bar{P} - \bar{P}_0)/\sigma$  may be thought of as a quantity like a difference of two concentrations, it is closer than a pigmentation frequency to the sort of variable on which gene substitution might be expected to have an equal additive effect throughout the skin. If this were so, the  $(\bar{P} - \bar{P}_0)/\sigma$  values

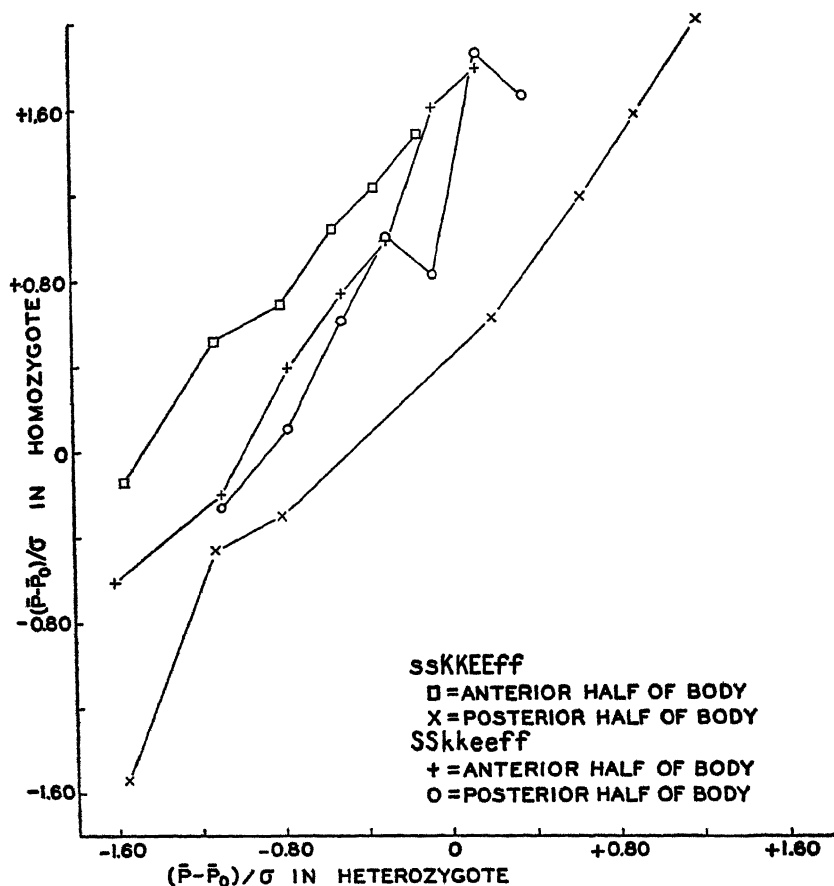


FIGURE 7.—Relation between transformed pigmentation frequencies of a heterozygous spotted type and the corresponding homozygote.

for a series of points in the homozygote, plotted against the values for the same points in the corresponding heterozygote, ought to fall along a straight line of slope 1. The transformed data of table 1 have been plotted in this way in figure 7 (the values for forelegs and anterior dorsum have been grouped together, as have the values for hind legs and posterior dorsum).

Three general relations seem to be indicated by figure 7:

(1) Within either the anterior or posterior half of the body the  $(\bar{P} - \bar{P}_0)/\sigma$

values of *ssKKEEff* (35%) do fall along a straight line of slope 1 against the corresponding values in *ssKkEeff* (35%  $\times$  100%). That is, there is a constant increment from heterozygote to homozygote as though *K* and *E* had a simply additive effect; but

(2) The constant increment within the anterior half of the body is much greater than the increment within the posterior half; the values are approximately 1.6 and 0.6. (There is some indication of an even higher increment on the head proper than on the forelegs and anterior dorsum.)

(3) The  $(\bar{P} - \bar{P}_0)/\sigma$  values of *SSkkee* (20%) fall along approximately the same straight line against the *Sskkee* (20%  $\times$  100%) values for both halves of the body, but the line seems to have a slope greater than 1. This is as though *S* had some sort of multiplicative effect. Here again a difference in nature of primary action between *S* and *KE* is indicated.

*Correlations.* From a comparative study of spotted mammals and birds ALLEN (1914) concluded that in both classes the skin consists dorsally of eleven contiguous or slightly overlapping cell groups within each of which, at some stage of development, a substance or process necessary for subsequent pigment formation spreads from the center towards the edges; that the extension occurs independently in the different areas and to its full extent in animals which become unspotted; that where the extending process stops short of the edge of its area, the peripheral territory becomes unpigmented; and that the spotting pattern of an individual is identical with the extent of spread of the determining process from the centers of the eye, ear, scapular, lumbar and rump areas of each half of the body.

ILJIN (1928) reached a different conclusion primarily from correlation studies on spotted guinea pigs: that spotting patterns are determined, not by restriction of a process which occurs in unspotted animals, but by extension of an action found only in spotted individuals; that in the mouse this action spreads from each of twelve "points of depigmentation" on the dorsal surface; and that between some of these points there is a correlation in degree of extension, the magnitude of correlation depending on the distance between the points considered.

ILJIN's "points of origin of depigmentation" coincide very closely with the margins of, or boundaries between, ALLEN's pigmentation areas. Both are located, in the main, at the common low points of the present pigmentation frequency fields, that is on the toes and tail tip, nose tip, center of forehead and anterior lumbar region.

ILJIN assumed depigmentation centers at front and rear of the third-quarter of the dorsum; ALLEN, a pigmentation center in about the middle of this quadrant. Now let two points be considered, one near the front, one near the rear of the third quadrant. If both points bear unpigmented fur in a given mouse it is indicated, according to ILJIN, that one has not

been reached by a process spreading from the anterior center, the other by a process from the posterior center. According to ALLEN, neither has been reached by one process diffusing from a single center. Now this quadrant of the dorsum corresponds to the series of points from 5 through 10 in table 3 among which most pairs of points show a reasonably large pigmentation association. These associations would mean peripheral correlation within one spreading process according to ALLEN, correlation between two processes under ILJIN's scheme. The former is at least simpler, though not necessarily more probable; it is equivalent to supposing that any one extending process tends to spread nearly equally in all directions; that the variation in extent is like the variation in aperture of an iris diaphragm rather than like an irregular, amoeboid variation in outline.

But ALLEN's scheme meets a certain amount of difficulty in the data of table 3. Point 9 has been shown never to be pigmented without pigmented fur extending from there to the posterior margin of the rump, which would mean that the last quarter of the dorsum lies in the rump field. But the pigmentation response of point 9 is positively associated also with that of points as far forward as the center of the dorsum (point 5), which would seem to mean that points 5 and 9 lie in ALLEN's lumbar field. The same difficulty is met at point 2, which seems to lie in both scapular and lumbar fields.

One possible solution is suggested by those skins which show "islands" or "peninsulas" of pigmented fur surrounded by colorless fur (figure 1). Under ALLEN's hypothesis these are cases where the spreading processes of three adjacent fields have failed to meet at their two borders. They may be found in three general positions centering approximately at points 1, 5 and 9 of table 3; that is, at the anterior end of the second quadrant, between second and third quadrants, and at the posterior end of the third quadrant. The three types of island are not sharply demarcated, but they do have a modal tendency. The first and third correspond to restriction of ALLEN's shoulder and lumbar fields, respectively. But the second is at the boundary between these two fields, and so might be supposed to define another pigmentation center ("mid-dorsal") not indicated by ALLEN, covering part of the territory of his scapular and lumbar areas. Within this common region, it might be supposed that pigmentation is sometimes determined by the spreading process from one center, sometimes by that of the other center, or perhaps by both jointly. One further alteration in ALLEN's scheme would be necessary from the present data. Since pigmentation on the foreleg does not occur without pigment on the ear, the former must belong to the ear field, rather than to the scapular as ALLEN supposed. Thus, if overlapping local fields are involved at some stage in the

embryogeny of spotting pattern, they perhaps number six on each side of the body: eye, ear, scapular, mid-dorsal, lumbar and rump.

In the absence of any direct demonstration the pigmentation fields of ALLEN and ILJIN, inferred from the regularities in location of pigmented and white fur areas, and the "concentration gradients," inferred from the regional variation in pigmentation frequency, are both merely ways of restating the primary data of genetic and non-genetic variation in spotting patterns. Each suggests that the next step in the analysis of the developmental basis of spotting patterns is a search for conditions which vary systematically throughout the embryonic skin (and also to a certain extent from individual to individual), for example, time of attaining some particular stage of morphological differentiation, rate of growth, etc. Some such pattern seems almost certainly involved, determining that in some regions of the skin cells containing several mutant (perhaps inactive) genes can form pigment whereas in other regions of the skin the whole wild-type gene set must be present. Regarded in this way the formation of mammalian spotting patterns is a relatively superficial and accessible analogue to the more fundamental ontogenetic processes by which genes and cytoplasmic differentials, tracing back ultimately to the structure of the fertilized egg, together determine the location of morphological elements more important than hair pigment. At least from this aspect they deserve further study.

#### THE PHYSIOLOGICAL DIFFERENCE BETWEEN COLORED AND WHITE AREAS

The question has frequently arisen what component of the reaction system leading to presence of pigment varies in the skin of a spotted mammal. ONSLOW (1915) and BLOCH (1927) each reported the white-furred skin regions of spotted mammals to lack a melanogenic enzyme which is present in the colored-furred areas and throughout the skin of unspotted animals. ONSLOW identified the enzyme as tyrosinase (rabbits, mice); BLOCH, as a more specific "dopa oxidase" (guinea pigs). The latter has since come to be rather generally accepted as the melanogenic enzyme of mammals.

FUGH (1933) reported that tyrosinase can only seldom be found even in extracts of all-black skin. In about 15 experiments she found no evidence of tyrosinase activity even when ONSLOW's procedure was followed as closely as possible. But in a further set of five extractions, two did produce a grey coloration in tyrosine solution after 30-33 hours. She concluded that black rabbit skin does contain a true tyrosinase.

BLOCH (1917, 1927) reported that frozen sections of skin, immersed in a 0.1% solution of dioxyphenylalanine pH 7.3-7.4, in 6-12 hours show formation of additional pigment granules *only* in those places where

melanin had previously formed naturally—in the melanoblasts. Among a number of mono- and di-hydric phenols only dioxyphenylalanine gave the reaction. On this basis BLOCH concluded that mammals, unlike great numbers of invertebrate and plant species, do not contain a tyrosinase but have another pigment forming enzyme which is substrate specific to dioxyphenylalanine.

BLOCH found the dopa-oxidase reaction of sections of white-spotted guinea pig skin to be negative in the follicles producing white hairs and positive in those forming pigmented hairs. But PRZIBRAM, DEMBOWSKY and BRECHER (1921) have suggested, from evidence that regions in active melanogenesis are more alkaline than the surrounding tissue, that the dopa reaction is identical with the spontaneous oxidation which dopa undergoes in vitro at alkalinities not much great than pH 7.5.

Hence so far as can be told from the published material, there exists an anomalous situation with respect to the physiological differences between colorless and pigment-forming follicles of spotted mammals: an enzyme difference is reported by each of two experimental methods, but doubt is cast on the validity of each. If PUGH's finding is correct that even with ONSLOW'S extraction method only about two out of seventeen samples from black skin show tyrosinase activity, then ONSLOW'S results may have been accidental; if (as is not clear from his report) he tested only one or two white skin extracts, the failure of tyrosinase activity in these cases may have been only an instance of the same random failure which PUGH observed even in black extracts. And BLOCH'S report of a dopa-oxidase difference between colored and unpigmented follicles in frozen sections may have been non-enzymic depending only upon the reported greater alkalinity of the pigment-producing follicles.

Accordingly it has seemed desirable to re-examine the existence of an enzyme difference between colored- and white-furred areas of piebald mouse skin. Because regular results could not be secured in preliminary tests with skin extracts and dopa, tyrosinase was used as a substrate in further study which was carried out as follows.

Comparable tests were made of the tyrosinase activity of extracts of skin of black unspotted mice and of extreme spotted, "all white," mice (DUNN and CHARLES 1937) only few of which have any pigmented hairs. Groups of 8-12 animals 2-4 days old were decapitated and bled as thoroughly as possible. The skins were removed with minimal connective tissue adhering and ground in a glass mortar with sand, chloroform, water or Ringer solution and pH 7.4 phosphate buffer (the amounts are shown in table 4). The resulting fairly homogeneous, gelatinous, grey or pale pink paste was centrifuged and filtered through a Buechner funnel. The opalescent filtrate was divided among a number of test tubes to which were



TABLE 4  
*Preparation of skin extracts for tyrosinase tests.*

| EXTRACT<br>NUMBER | COLOR<br>OF SKIN | NUMBER<br>OF SKINS | EXTRACTION IN |         |         |                   |
|-------------------|------------------|--------------------|---------------|---------|---------|-------------------|
|                   |                  |                    | pH 8 BUFFER   | WATER   | RINGER  | CHCl <sub>3</sub> |
| I.                | black            | 8                  | 1.5 cc.       | —       | 3.5 cc. | 0.5 cc.           |
| II.               | albino           | 8                  | 1.5 cc.       | —       | 3.5 cc. | 0.5 cc.           |
| III.              | black            | 11                 | 1.5 cc.       | —       | 3.5 cc. | 0.5 cc.           |
| IV.               | black            | 10                 | 1.5 cc.       | —       | 3.5 cc. | 0.5 cc.           |
| V.                | black            | 12                 | 5.0 cc.       | —       | —       | 5.0 cc.           |
| VI.               | white*           | 12                 | 5.0 cc.       | —       | —       | 5.0 cc.           |
| VII.              | black            | 9                  | 2.0 cc.       | 3.0 cc. | —       | few drops         |
| VIII.             | white*           | 9                  | 2.0 cc.       | 3.0 cc. | —       | few drops         |

\* "All white" extreme spotted.

added substrate as shown in table 5 and a few drops of chloroform. Tubes were kept either at room temperature or at 38°C. as indicated, and observed after intervals varying from 12–24 hours. The results are shown in table 5.

In the first series tyrosinase activity, without peroxide and so meeting the criteria for a true tyrosinase, was found in the extracts of two out of three groups of black skins (I and III). The degree of melanization after 20

TABLE 5  
*Tyrosinase activity of skin extracts.*

| EXTRACT<br>NUMBER        | COLOR  | TEMP.<br>(°C.) | TIME<br>(HRS.) | 1 CC.<br>H <sub>2</sub> O        | 1 CC. DOPA<br>0.04%  | 1 CC. TYRA-<br>MINE 2.02% | 1 CC. TYRO-<br>SINE 0.04% |
|--------------------------|--------|----------------|----------------|----------------------------------|----------------------|---------------------------|---------------------------|
| H <sub>2</sub> O         | —      | 20±            | 17             | —                                | ++                   | —                         | —                         |
| I.                       | black  | 20±            | 17             | —                                | ++++*                | —                         | +++                       |
| II.                      | albino | 20±            | 48             | —                                | +++                  | —                         | —                         |
| III.                     | black  | 38             | 19             | —                                | +++                  | —                         | +++                       |
| III.                     | black  | 38             | 19             | —                                | +++                  | —                         | —                         |
| (diluted 1:5 with water) |        |                |                |                                  |                      |                           |                           |
| IV.                      | black  | 20±            | 21             | —                                | +++                  | —                         | —                         |
| IV.                      | black  | 20±            | 21             | —                                | ++                   | —                         | —                         |
|                          |        |                |                | p-cresol<br>catechol<br>aniline† | p-cresol<br>catechol | p-cresol<br>aniline       | p-cresol                  |
| V.                       | black  | 20±            | 24             | —                                | ++‡                  | +                         | —                         |
| VI.                      | white  | 20±            | 24             | —                                | —                    | —                         | —                         |
| VII.                     | black  | 20±            | 20             | —                                | ++                   | +                         | —                         |
| VIII.                    | white  | 20±            | 20             | —                                | —                    | —                         | —                         |

\* +++ indicates deep gray color through top half of solution when tyrosine is substrate, throughout when dopa is used.

† 1 cc. 0.1% p-cresol; 0.1 cc. 0.1% catechol; 0.5 cc. 1% aniline.

‡ ++ indicates medium brown color; +, reddish tan.

hours was considerably less than that reported by ONSLOW who found a charcoal black ring at the top of his test solutions, but was comparable to that secured by PUGH.

A third extract (IV) of black skin did not give any evidence of tyrosinase activity, either full strength or diluted. This same inactivity was found in extract III diluted 1:5 with water, although the undiluted extract was active. The fact that in this case at least dilution led to loss of tyrosinase activity suggests that in IV, where the method was apparently comparable some unknown difference in procedure led to lower concentration of enzyme in the extract as PUGH suggested in reference to the irregularity of her results. It is of interest in this connection that PUGH (1933) reported the same unexplained effect of dilution on the tyrosinase activity of mealworm extracts.

The difficulty in using dopa, under simple conditions, as a substrate for testing the presence of melanogenic enzymes in skin extracts is indicated in table 5 by its reaction in all cases, even without addition of other substances.

In an attempt to minimize the number of reactions involved in the tyrosinase tests, p-cresol was tried as a substrate known to be oxidized by tyrosinase without undergoing the complex series of secondary reactions which occur when tyrosine is used. As a possible means to increase the sensitivity of these further tests catechol and/or aniline were also added, in view of RICHTER's report (1934) that (1) the direct action of tyrosinase is upon o-dihydric and not monohydric phenols, forming o-quinones, and that monohydric phenols serve as substrates for tyrosinase only after reacting with o-quinone to form dihydric phenols: and (2) that higher concentrations of o-quinone inhibit the enzyme. Hence catechol was added as a priming agent (that is, to supply o-quinone) in concentration so low that its own oxidation would not add enough color to the solution to suggest tyrosinase activity. This concentration should still be high enough to furnish adequate substrate for the initial action of the enzyme, if present. The aniline was added to combine with any possible, though unlikely, excess of o-quinone which might be formed.

Two black skin extracts tested under these conditions both showed the presence of tyrosinase (V and VII), with and without aniline. When catechol was omitted, only one of the extracts showed activity presumably because of the lack of enough priming material like catechol in the skin extract.

Two extracts from the skin of extreme spotted animals (VI and VIII), each tested simultaneously with one of the black extracts, did not show evidence of tyrosinase activity under any test conditions.

Since only one out of the total of five black extracts tested failed to react

positively for tyrosinase, the chance that two samples from other skin of similar enzyme content should both fail for the same unknown reason as the black is only about one in twenty-five (that is, the product of the chances that one should fail). Hence it may be concluded that probably, as ONSLOW reported originally, the white-furred areas of piebald mouse skin either lack, or have a much lower concentration of, the melanogenic enzyme present in the skin of colored animals. The data definitely confirm PUGH's finding that the enzyme is a true tyrosinase in that it operates without added peroxide and that, contrary to BLOCH's conclusion, mammals (or at least mice) thus do not lack the potentially melanogenic enzyme present in invertebrates. That this tyrosinase lies at least partly in the melanoblasts of the hair follicles and is actually the melanogenic enzyme of those cells is not proved by the present results, although it seems likely from the probable deficiency of the enzyme in the regions of skin where the follicles are not producing melanin. It is also yet to be proved that the colored-furred areas of spotted mouse skin have the same concentration of tyrosinase as the skin of unspotted mice, although this seems likely from the identity of the pigmentation. And it remains uncertain whether the white-furred areas of spotted mice are completely lacking in tyrosinase.

## SUMMARY

The patterns of white-spotted mice have long been known to be rather diverse, even within presumably almost isogenic strains. The variability is limited, however; some regions of the skin bear pigmented fur in every individual; other regions always bear white fur, and it is only the intervening areas that have a variable behavior which may be characterized by the proportion of cases in which the hairs are pigmented.

1. Counts have been made of the proportion of animals bearing pigmented fur on each of 508 very small skin areas, in the presence of each of six combinations of "spotting" genes.

2. The proportions so obtained ("pigmentation frequencies") vary systematically over the skin surface, in the presence of any one genotype, forming a gradient field which drops off from the ear and rump regions towards the feet, tail tip, and mid-lumbar region.

3. The frequency fields of *ssKKEEFF* (4% of white fur), *ssKKEEff* (35%), *ssKkEeFf* (hybrid between 4% and 100% white strains) and *ssKkEeff* (35%  $\times$  100%) are found to have the same general form but consistently different heights, the frequencies diminishing in the order indicated.

4. Similarly the frequency field of *SSkkeeff* (20%) is consistently higher than that of *Sskkeeff* (20%  $\times$  100%); but neither of these two falls into the

previous series; that is, both have a somewhat different form than the fields obtained in the presence of *ss*, lower in the anterior dorsum and head, higher on the posterior dorsum and legs.

5. The difference in shape of pigmentation frequency field between genotypes containing *KEF* without *S* and those containing *S* without *KEF* is taken to indicate that *KEF* and *S*, though both acting ultimately on pigmentation frequency, have different sorts of primary effect.

6. The increase in pigmentation frequency of a given point from heterozygote to homozygote (*Sskkeeff* to *SSkkeeff*; *ssKkEeff* to *ssKKEeff*) is found to vary complexly with the value in the heterozygote.

7. But if the pigmentation frequencies are subjected to an inverse probability transformation (WRIGHT 1920) the values in heterozygote and homozygote are found to have an approximately linear relation. The transformation does not bring about an approach to constant difference between *Sskkeeff* and *SSkkeeff*, although it does for *ssKkEeff* and *ssKKEeff*, which is taken to be a second indication of a difference in nature of primary effects between the (*KEF*) and *S* genes.

8. Following WRIGHT (1920) the frequency fields are considered in terms of some hypothetical material whose concentration *P* at a particular point of the skin must equal or exceed a minimal value *P*<sub>0</sub> for the ultimate formation of pigment. Most aspects of the observed regional and genotypic variation of pigmentation frequency can be accounted for if it is assumed that:

(a) the *P* of a particular skin point varies somewhat from animal to animal of the same genotype;

(b) the average value either of *P*, or of *P*<sub>0</sub> or of both, in the presence of a given gene combination, varies systematically from point to point over the skin surface;

(c) *K*, *E*, *F* have a constant additive effect on either *P* or *P*<sub>0</sub> throughout the anterior and posterior halves of the body, the anterior increment being nearly three times as large as the posterior;

(d) *S* has some sort of multiplicative effect on one of the two variables, *P* or *P*<sub>0</sub>.

9. As reported by ONSLOW (1915) but somewhat doubted from the results of subsequent workers, tyrosinase can generally be detected in extracts of skin which is forming pigmented hairs; but it is either absent, or greatly diminished, in extracts of those skin regions of spotted mice which are forming unpigmented hairs.

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# BAR-EYED MOSAICS IN *DROSOPHILA MELANOGASTER*

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Received June 7, 1938

FOR investigating certain aspects of the general problem of gene action it would be a great help if it were possible to substitute a gene for one of its alleles at the 2-cell stage, at the 4-cell stage, and so on, successively, at known later stages during development. The mere statement of such a hopeless ideal makes it at once clear that by such a method we could obtain information on the time during development when a gene is effective in producing any of its known effects, on the duration of its action, and on the rate at which it acts at every stage of any process in the developmental nexus which it affects.

Of the methods available at present for the study of gene action there are at least two which make an approach to this general method of gene substitution during development; namely, transplantation, in those cases where the implant and the host differ in some one or a few genes, as in the studies of BEADLE and EPHRUSSI (1937), and secondly, the use of mosaics, which are in effect spontaneous orthotopic transplants that avoid the distorting effects of an operation.

Of these methods each has its distinct advantages and drawbacks. The method of transplantation is especially suitable for studying the transport of materials from the organism to a part and in detecting the contribution of the part to the rest of the organism, and so gives promise of the eventual isolation and chemical identification of the diffusible substances involved. While the time relations can be known by this method, the results have not as yet led to a knowledge of the time course of any reaction in quantitative terms. On the other hand the method of analysis by the use of mosaics does supply quantitative data in suitable material, but the time relations, at least for spontaneously produced mosaics, are still not precisely known. An account of the results obtained by these two methods, with full reference to the literature is included by GOLDSCHMIDT (1938) in his recent comprehensive exposition of the facts and their implications, relating to the whole subject of physiological genetics.

The present paper is concerned with white-eyed mosaics in the bar series of *Drosophila*. Previous reports on the mosaic condition of the eye of *Drosophila melanogaster* are those of BRIDGES (1925), STURTEVANT (1927) and HERSH (1934) in which the mosaic condition was produced by the use of Minute-n. PATTERSON (1929) who produced eye mosaics by x-raying larvae at different ages showed that the facet-producing reaction in the

wild type eye extended practically throughout the entire egg-larval period, a result which has been confirmed by MARGOLIS and ROBERTSON (1937) in their determination of the temperature effective period for the wild type eye. HASKINS (1935), whose main interest was in the determination of the genic volume at the white locus, obtained mosaics by x-raying larvae at age 18 to 24 hours and concluded that at that age there are about 20 cells in the eye anlage.

The data on white bar-eyed mosaics presented below were obtained by the use of Minute-n. Mosaics from matings of  $fBMn/fB$  ♀  $\times w$  ♂ ♂ were recovered at 20°, 25° and 30°C, and in addition mosaics from  $fBMn/fB$  ♀  $\times wBB$  ♂ ♂ matings were obtained at 25°C. In these experiments the flies were otherwise roughly isogenic, but not strictly so, since there was no prolonged period of suitable crossing and inbreeding of the stocks previous to doing the experiments.

The fluctuations from the temperatures mentioned above were less than a degree in either direction. The usual variation of the incubators was about 0.7° either way, except that in the experiments involving ultrabar at 25°, the temperature rose to nearly 27° for a period of about 24 hours.

The data on nearly 20,000 flies are summarized in table 1, where it can be seen that the number of mosaics among the  $Mn$  females is about 1 percent or less; more precisely 0.97 at 20°, 1.15 at 25° and 0.42 at 30° in the mosaics among the  $fBMn/w$  females. Among the  $fBMn/wBB$  females raised at 25° there were found 18 mosaics in a total of 1728 flies, or 1.04 percent. But this may be misleading, since in this series 525  $Mn$  females were recovered before the first mosaic was found; 1310  $Mn$  females were examined before the fifth mosaic was in hand. Four of the total 18  $Mn$  mosaics in this series were found among the progeny of the same female which incidentally also produced two gynandromorphs (not included in table 1). Taking these details into account, we might estimate that the percentage rate of occurrence of  $Mn$  mosaics in this series which it is fair to compare with the results of the other series is perhaps more nearly about 0.5 percent rather than the 1.04 percent given directly by the data. Furthermore, it was in this series that the temperature rose to 27° for about 24 hours.

The non- $Mn$  mosaics that appeared among the  $fB/w$  flies comprised 0.18 percent of the non- $Mn$  females at 20°, and 0.32 percent at 30°. Unfortunately, no record was made of the non- $Mn$  mosaics among the flies of similar genetic constitution raised at 25°, since at the time these flies were raised, the elimination of the  $Mn$  chromosome was accepted as the mechanism of mosaic formation instead of somatic crossing over which STERN (1936) has shown to be a much more likely explanation. Non- $Mn$  mosaics among the  $fB/wBB$  flies raised at 25° comprised only 0.08 percent

of the total; 2 out of 2626 (table 1). Consequently, the presence of *Mn* increased the frequency of mosaics (somatic crossing over) from 4 to 10 times over that found in the flies of similar constitution but which lacked the *Mn* factor.

The data allow no very certain conclusion to be drawn in regard to the effect of temperature on somatic crossing over, although there seems to be a distinct tendency for the rate to be lower at 30° than at 20° or 25°. PLOUGH (1921) found that 31.5° caused little or no change on gametic crossing over in any part of the X chromosome. STERN and RENTSCHLER (1936) found in experiments involving *Mn* a lower rate of somatic crossing over at 30° than at 17° and 25°C.

The data on facet number of the mosaic-eyed flies are given in table 2. Before discussing the quantitative aspects of the data it might be men-

TABLE 1

*Summary of the data. The first three columns of data give the numbers of offspring from matings of fBMn/fB ♀ × w♂ ♂; the last is for the cross fBMn/fB ♀ × wBB ♂ ♂.*

|                        | 20°  | 25°  | 30°  | 25°  |
|------------------------|------|------|------|------|
| Minutes                | 2155 | 1286 | 1423 | 1710 |
| Non-Minutes            | 2835 | 1730 | 1894 | 2624 |
| <i>Mn</i> mosaics      | 21   | 15   | 6    | 18   |
| Non- <i>Mn</i> mosaics | 5    | —    | 6    | 2    |
| fB males               | 2373 | 1462 | 861  | 2301 |

tioned that mosaic spots of forked bristles and abnormally pigmented abdomens were noticed from time to time but no systematic study was made. In respect to the eyes no flies were found in which both eyes showed the mosaic condition. The right and left eyes were equally affected. In the mosaic eye the white patch was a larger or smaller segment that extended to the margin of the faceted area. There was not a single specimen in which the white area was entirely surrounded by red facets. STURTEVANT (1927, figure 4), however, has found that such a condition does occur. The larger the patch of white facets, the greater seems to be the distortion in shape of the faceted area over what is typical for bar, variable as bar eye itself is. Although distorted, the larger mosaic eyes tend to approach the wild type in outline. In two notable specimens (table 2) in which the "mosaic" eye was entirely white but somewhat smaller than wild type, the eye was actually oval in outline. This raises a point in regard to the qualitative effect in the bar series, namely, the indentation on the anterior margin of the faceted area. The conclusion seems obvious that the developmental antecedents of this indentation are not produced in the early stages of development.

With but two exceptions, found among the *fBMn/wBB* females, the



mosaic eye was always a single unified area. In one of these there was a group of 5 white facets on the ventral border, separated about 1 mm from the rest of the faceted area. The other exception was red-and-white banded. Beginning with the dorsal border of the eye and proceeding ventrally this specimen showed the following order of red and white facets: 1 white, 8 red, 8 white, 14 red, 3 white and 2 additional red facets along the edge of

TABLE 2

*Facet number for the mosaics listed in table 1. The first three columns are for the mosaics from matings of  $fBMn/fB \text{ } \varnothing \times w\bar{c}\bar{c} \text{ } \sigma$ , the last is for those from the cross  $fBMn/fB \text{ } \varnothing \times wBB \text{ } \sigma \text{ } \sigma$ . For each mosaic there is given first the total facet number in the mosaic eye, followed in parentheses by the number of white facets, which in turn is followed by the total facet number in the opposite eye. For example, 203 (1) 189 indicates that in the mosaic eye were 203 facets 1 of which was white; in the opposite eye there were 189 red facets. In the last two columns the data below the cross-bars are for non-Mn mosaics.*

| 20°   |         |       | 25° |       |     | 30° |       |     | 25° |      |    |
|-------|---------|-------|-----|-------|-----|-----|-------|-----|-----|------|----|
| 203   | (1)     | 189   | 135 | (4)   | 126 | 93  | (14)  | 87  | 25  | (11) | 26 |
| 238.5 | (7)     | 199.5 | 137 | (9)   | 118 | 133 | (41)  | 78  | 28  | (1)  | 28 |
| 241   | (8)     | 197.5 | 138 | (8)   | 104 | 300 | (143) | 190 | 28  | (2)  | 32 |
| 247.5 | (19.5)  | 210   | 138 | (12)  | 111 | 310 | (7)   | 185 | 28  | (13) | 34 |
| 249   | (39)    | 312   | 149 | (2)   | —   | 537 | (537) | 103 | 31  | (2)  | 32 |
| 264   | (24.5)  | 263.5 | 149 | (3)   | 140 | 602 | (455) | 117 | 31  | (23) | 31 |
| 267   | (9)     | 277.5 | 156 | (21)  | 110 |     |       |     | 32  | (3)  | 33 |
| 275.5 | (18)    | 314.5 | 164 | (4)   | 152 | 92  | (3)   | 108 | 34  | (9)  | 27 |
| 300   | (6)     | 296.5 | 172 | (2)   | 142 | 111 | (4)   | 78  | 36  | (9)  | 36 |
| 302   | (64.5)  | 208.5 | 181 | (6)   | 146 | 131 | (8)   | 128 | 36  | (12) | 33 |
| 314   | (105.5) | 215   | 197 | (55)  | 131 | 132 | (29)  | 105 | 36  | (31) | 35 |
| 325   | (60)    | 276   | 206 | (24)  | 170 | 216 | (2)   | 108 | 37  | (6)  | 32 |
| 335   | (11.5)  | 323   | 267 | (60)  | 179 | 544 | (62)  | 219 | 37  | (11) | 30 |
| 339   | (7)     | 303   | 286 | (84)  | 135 |     |       |     | 39  | (8)  | —  |
| 398.5 | (146.5) | 312   | 306 | (113) | 156 |     |       |     | 40  | (39) | 35 |
| 403   | (109)   | 270.5 |     |       |     |     |       |     | 41  | (13) | 36 |
| 410.5 | (106)   | 295.5 |     |       |     |     |       |     | 42  | (16) | 38 |
| 448   | (20.5)  | 419   |     |       |     |     |       |     | 45  | (12) | 42 |
| 483.5 | (263.5) | 338   |     |       |     |     |       |     |     |      |    |
| 500   | (500)   | 300   |     |       |     |     |       |     | 36  | (17) | 37 |
| 619   | (393)   | 197.5 |     |       |     |     |       |     | 44  | (8)  | 34 |

the band of 8 white facets, giving a total of 12 white and 24 red facets in the mosaic eye. The opposite eye of this specimen possessed 33 red facets.

STURTEVANT (1927) reported that in the mosaics found by him among *BBMn/w* females, the number of red facets in the mosaic eye is greater than that found in the normal eye of the same fly. This is the case also for the mosaics found by us among the *fBMn/w* females raised at 25°. The difference in number of red facets between the mosaic and the non-mosaic eye ranges from 5 to 67, with an average of 22 facets in favor of the mosaic eye. The same relationship was not found in the other three sets of mosaics

reported here (see table 2). For example, in the mosaics among the *fBMn/w* females raised at 20° sometimes the mosaic eye had a larger number of red facets, and sometimes the non-mosaic eye had the larger number of red facets. The average difference, about 10 facets, is in favor of the non-mosaic eye. The other two series of mosaics depart still more widely in this respect. It seems clear that the greater number of red facets in the mosaic eye compared to the non-mosaic eye of the opposite side, commonly found in the *BMn/w* and *BBMn/w* mosaics at 25°, is merely a special case of some more general relationship. To give much importance to the larger number of red facets in the mosaic eye at 25° would be perhaps to regard standard conditions at 25° as a base of reference favored by nature for revealing the fundamental principles of genic action. What is obviously needed is a more comprehensive view based on a study of the quantitative relations exhibited by adequate data. But it is, nevertheless, clear that when the mosaic eye has a larger number of red facets than the opposite eye, then facets are formed in bar tissue that would not have had them otherwise (STURTEVANT 1927).

The facet counts of the individual mosaics (table 2) show, in spite of the variability, that with increasing size of the mosaic eye, the number of white facets increases, but it is also clear that the increase cannot be regarded as due simply to the white facets. As pointed out above, sometimes the number of red facets in the mosaic eye is greater than in the opposite eye and sometimes not.

In the attempt to find some simple uniformity in the data, it was discovered that when the number of white facets ( $y$ ) is plotted logarithmically against the total number of facets ( $x$ ) in the mosaic eye, the data for each series fall within a straight band which conforms to the simple power function

$$y = bx^k$$

known to biologists as the relative growth function or the equation of allometry, but it will perhaps do no harm to point out that power functions are among the most common relations found in nature.

The slope of the band which gives the value of  $k$  is about 5 in each case, but the band is shifted toward the left as the order of magnitude of eye size for any set of mosaics decreases. In other words, the value of  $b$  increases in flies of genetic constitution for smaller eye size and for those raised at higher temperatures, which in the flies under discussion also reduce the eye size.

For figure 1 the data for the two sets of mosaics raised at 25° and the one raised at 20° given in table 2 were seriated in suitable size classes on the basis of total facet number in the mosaic eye. This procedure gave the

points plotted on the graph, so that each point in the figure represents usually the average of from 3 to 6 mosaics. However, several of the points, especially those for large eye size, represent single specimens. The fitted lines of the figure were calculated by the method of averages, and are ex-

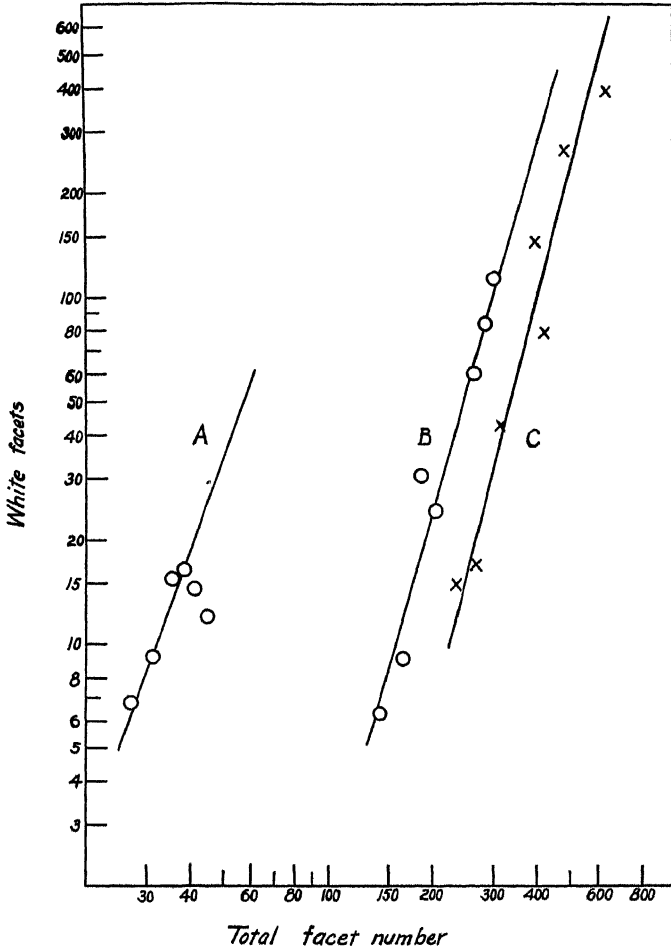


FIGURE 1.—The number of white facets ( $y$ ) plotted logarithmically against total facet number ( $x$ ) of the mosaic eyes; A,  $fBMn/wBB$  mosaics at  $25^\circ$ ; B,  $fBMn/w$  mosaics at  $25^\circ$ ; C,  $fBMn/w$  mosaics at  $20^\circ$ .

tended upward to the point where  $y = x$ , about which we shall have more to say below. (The point,  $x$ , 45;  $y$ , 12, which departs most widely from line A was not included in the calculations.) That the data conform to the power function implies that the increase in total facet number in the mosaic eye over the opposite eye is not due alone to the white facets. For certain portions of the curves conformity to the power function requires

a larger number of red facets in the mosaic eye than in the opposite eye and sometimes a smaller number. If the increase were due alone to the white facets it is clear that the data would not conform to the power function.

The calculated values of  $k$  are lower than the slopes determined graphically from the bands given by the plotted points for the individual specimens. The  $k$  value for the  $fBMn/wBB$  mosaics (fig. 1, A) is about 2.81. The value of  $k$  for the  $fBMn/w$  mosaics at  $25^\circ$  is 3.53 (fig. 1, B) and 3.86 at  $20^\circ$  (fig. 1, C). The values of  $\log b$  for curves A, B, C are, respectively,  $-3.238$ ,  $-6.744$  and  $-8.044$ . These values can be considered only as approximately accurate.

Although the values of  $k$  differ it will be seen from figure 1 that the curves are roughly parallel to one another. This is due to the circumstance that the angles which the curves make with the x-axis are all in the neighborhood of  $75^\circ$ , a region where the tangent begins to show a relatively large change for a small increase in the angle.

The smaller the order of eye size in any set of mosaics, the greater is the shift of the curve toward the left. That is, the larger the value of  $b$  then the greater the shift of the curve to the left. It is clear from the figure that the main difference between these three sets of  $Mn$  mosaics is a difference in the constant  $b$  of the power function, but the value of  $k$  for the  $fBMn/wBB$  mosaics at  $25^\circ$  is lower than the  $k$ -values for the  $fBMn/w$  mosaics at  $20^\circ$  and  $25^\circ$  which are closely similar to each other.

On the purely mathematical side  $k$  is the ratio of the percentage increase in  $y$  to the percentage increase in  $x$ . If we take the value of  $k$  as about 4, then in biological terms the number of white facets increases about 4 times over that of the total eye. Consequently we soon come to a value of the function when  $y=x$ , that is, at this point, the eye will be entirely white. It can readily be shown that this point of the curve is given by the relation

$$\log y = (\log b / 1 - k).$$

The value when  $y=x$  for the  $fBMn/w$  mosaics (fig. 1, B and C) is 464 facets at  $25^\circ$  and 656 facets at  $20^\circ$ . These values are of course approximately average values. If we estimate the range of variability from the width of the bands obtained by plotting the mosaics individually, then the value of the function when  $y=x$  is from about 375 to 525 at  $25^\circ$  and from about 500 to 700 at  $20^\circ$ . In the set of  $fBMn/w$  mosaics at  $25^\circ$  none was found in which the eye was entirely white, but in the  $20^\circ$  series a remarkable specimen was found in which the "mosaic eye" was entirely white, having 500 facets; the opposite eye had 300 red facets.

For further discussion of the data in biological terms, it is necessary to make an assumption in regard to the relation between flies at different

points of the curve. The most direct and perhaps simplest assumption in this respect is that the greater the number of white facets then the earlier the event (somatic crossing over) occurs which changes the genetic constitution of certain cells and their descendants in the optic disk. This assumption is plausible on general grounds and fortunately it also has direct experimental evidence from full eye to support it. PATTERSON (1929) found that the younger the larvae were when treated with x-rays the larger on the average was the white-faceted area. In his experiment the change was only in genes for eye color.

In the *Mn* mosaics the change in genetic constitution for eye color denotes also a change at the same time in genetic factors for eye size at the bar locus. If somatic crossing over occurs to the left of *BMn* in *fBMn/w* females then there is no change in the genetic constitution at the bar locus although some cells will have the constitution for white facets *wfBMn/w* in an optic disk which otherwise is *fBMn/w* in constitution, and so will produce red facets. For a change in the genetic constitution at the bar locus the somatic crossing over must be to the right of *Mn* (we may neglect double crossing over). Fortunately, for this aspect of the problem STERN and RENTSCHLER (1936) worked with characters which enabled them to conclude that 118 out of 120 cases were derived from crossing over to the right of *Mn*. In the light of their evidence we may regard the mosaic condition of the eye to be, in practically all cases, a consequence of crossing over to the right of *Mn*.

When crossing over occurs to the right of *Mn* in *fBMn/w* females, the resulting cells have the constitution *fBMn/fBMn* and *w/w*. The former cells presumably die from the lethal effect of homozygous *Mn*, leaving the cells for white facets and wild type eye size in an optic disk which is otherwise *fBMn/w* in constitution. The earlier this event occurs, the larger on the average will be the white spot in an eye which is itself larger than it otherwise would have been as shown by the non-mosaic eye of the opposite side. The value when  $y=x$ , 464 facets for the *Mn* mosaics at 25°, and 656 facets for those at 20°, gives the average size expected when the crossing over takes place at the latest possible time in development for producing an all white eye in *fBMn/w* females. If the event happens earlier the eye will, of course, be all white and be still larger and more nearly approach the size of the wild type, about 750 facets at 25°, about 850 to 950 facets at 20°. The main conclusion from these considerations is that the action of the bar factor up to the latest time for the occurrence of an all white eye is such as to depress the size of the eye by about 285 facets at 25° and by about 200 to 300 facets at 20°. But, furthermore, it may be considered that this latest possible time for the production of an all white eye which is smaller than the wild type by nearly 300 facets may be regarded as the

a larger number of red facets in the mosaic eye than in the opposite eye and sometimes a smaller number. If the increase were due alone to the white facets it is clear that the data would not conform to the power function.

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time of appearance of the first cell in the optic disk in heterozygous bar females, which in this case also contains *Mn*. This of course does not necessarily mean that in the wild type at the same corresponding time of development there are a sufficient number of cells already present in the optic disk to differentiate later into about 300 facets. It may be taken to mean only that the difference at this time between wild type and heterozygous bar, projected forward as it were and stated in terms of completed development, has a value of about 300 facets.

What the actual processes are which bring about the difference remain of course to be discovered, but the most obvious suggestion would concern itself with localized differences in cell divisions in the optic disk, or in the cells whose descendants later form the optic disk. These may be differences in the time or date of the occurrence of mitotic cycles in this part of the body of the fly, as well as differences in their duration and in the rate at which they proceed. Such differences would necessarily need to be referred back to prior differences in the cellular physiological processes between the wild type and the members of the bar series. While the differences in the cellular physiological processes at the beginning of development would constitute the first links in the chain or nexus of causes leading from the genes to their effects, they may well be the last in order of discovery.

The third set of *fBMn/w* mosaics may be disposed of more briefly. At 30° only 6 *Mn* mosaics and 6 non-*Mn* mosaics were found among the progeny of the *fBMn/fB* ♀ ♀ × *w* ♂ ♂ crosses. Although the number is small yet the range in size both of the entire eye and of the mosaic spot is considerable (table 2). Assuming a conformity of the meager data to the power function, a graphic determination of the slope gives about 1.76 for the *Mn* mosaics and about 1.70 for the non-*Mn* mosaics, but the curve for the *Mn* mosaics is considerably to the left of that for the non-*Mn* mosaics, that is, the *Mn* mosaics give the larger value of the constant *b*. In this series a specimen with an all white eye containing 537 facets was found among the *Mn* mosaics; the opposite eye possessed 103 red facets. Apparently the usual distortion of developmental processes which takes place at supranormal temperatures comes to expression also in these mosaics, as shown by the low value of *k*.

In these three sets of mosaics from *fBMn/w* at 20°, 25° and 30° the white spot will have the constitution *w/w* in an optic disk which is otherwise *fBMn/w* in constitution, if we accept somatic crossing over to the right of *Mn* as the mechanism of mosaic production. These three sets thus agree in having a change in constitution from heterozygous bar to homozygous wild type at the bar locus, in other words, a genetic change for a larger eye.

The other set of mosaics which we still have to discuss is the complement



of the others in the sense that in these flies there is a change to a genetic constitution for smaller eye than that with which development of the egg started. That is, after somatic crossing over to the right of  $Mn$  in  $fBMn/wBB$  females we have  $wBB/wBB$  cells in an optic disk otherwise  $fBMn/wBB$  in genetic constitution. Consequently in the  $fBMn/wBB$  flies as the change in genetic constitution happens earlier and earlier we should expect to get relatively a larger and larger number of white facets but the whole eye would be expected to become smaller and smaller. The eye would be expected to change in size from that found in heterozygous bar ultrabar toward that found typically in homozygous ultrabar.

If there were no other condition acting to disturb in any way the simple operation of this process then a logarithmic plot of the number of white facets against total facet number in the mosaic eye should give a line of negative slope. However, when plotted the band of points for these mosaics has a positive slope ( $k$  is 5.2), while the value of  $k$  calculated from the grouped data, as mentioned above, is 2.81. Furthermore, the value is 61 facets at the point where  $y = x$ . But if, in this case, we expect on the basis of the mathematical relations to find a "mosaic" eye, all white containing 61 facets, we clearly draw an inference from the mathematical relations that falls beyond the limits allowed by biological fact. This follows, since flies of the constitution  $fB/wBB$  raised at  $25^\circ$  have a range in facet number from 36 to 47 with an average at  $40.8 \pm 0.37$  facets; in  $fBMn/wBB$  flies at  $25^\circ$  the average is  $36.0 \pm 0.43$  facets. If in the flies with the constitution  $fBMn/wBB$  there is a change in the optic disk during development to  $wBB/wBB$  the eye clearly would be expected to become smaller as the number of white facets increases, but the data in fact show that on the average the eye becomes larger. How is the apparent contradiction to be resolved? The explanation seems to be quite simple.

In the case of the  $fBMn/w$  mosaics the change in genetic constitution at the bar locus is one for a larger size of eye. If there were no variability in eye size, that is, if all flies of the given genetic constitution had the same size of eye, then the mosaic-eyed specimens among the  $fBMn/w$  females would be the only ones to differ in facet number. The mosaics would differ among themselves depending upon whether the somatic crossing over occurred earlier or later. But, of course, in actual fact the flies of any given genetic constitution show the usual variability. The variability in the mosaic specimens is superimposed upon the ordinary variability. As the change in genetic constitution happens earlier and earlier a larger and larger eye results which in time extends beyond the upper range of the ordinary curve of variability for flies with the constitution  $fBMn/w$ .

But in flies with the constitution  $fBMn/wBB$  when the change in genetic factors occurs the result is a constitution for smaller eye size. As

the somatic crossing over happens earlier and earlier the eye would be expected to become smaller and smaller but have a relatively larger number of white facets. Consequently, if the *fBMn/wBB* flies were the only ones which differed in facet number, then the smaller-eyed mosaics would have the larger number of white facets, resulting in a negative value of  $k$ . But the process of mosaic formation is superimposed upon the processes which produce the ordinary variability, and in this case practically within the limits of the ordinary curve of variability. The consequence is first to broaden the band within which the mosaics fall when plotted individually, and this in turn merely reduces the value of  $k$  below that found in the *fBMn/w* mosaics,—from nearly 4 to 2.81. The width of the band can be readily determined from the extreme values given by the two mosaics 25(11) and 45(12). Furthermore, the specimen in this series with 40 facets, 39 of which are white, is an indication that the change in constitution may happen early enough to give practically an all white eye yet still the eye may be on the upper limit of the range in total facet number. In this series the value  $y = x$  at 61 facets cannot be given biological meaning.

A different opinion might be advanced that the positive value of  $k$  in this case, where a negative value is expected, is a consequence of a growth stimulating influence of an intercellular nature that arises from the contact of tissues of different genetic constitution (see STURTEVANT 1927, also GOLDSCHMIDT 1938, p. 177). An examination of the *fBMn/wBB* mosaics (table 2, column 4) shows that in the mosaic eye the range is from 25 to 45 facets, while in the non-mosaic eye of the same flies the range is from 26 to 42 facets. The failure of the range downward to be reduced and the range upward to be perhaps slightly increased favors the view of an intercellular influence. But in any case the effect is quite small. It seems that the irregular fluctuating variability itself is sufficient to bring about a positive value of  $k$  in the *fBMn/wBB* mosaics.

The bar type of eye may be regarded as a form of localized dwarfism. Such marked defective departures on the minus side of the wild type or normal are usually called arrests in development, of which there are many instances but which need no extended discussion here. But it is clear that not all so-called developmental arrests are the end effects of the same essential type of defective process. We may distinguish two main types. There is first the type in which a normal anlage is produced with later a loss by atrophy or autolysis, as in the vestigial alleles (GOLDSCHMIDT 1935). In some cases the loss may be by an autotomy. The second main type of localized dwarfism would stand in contrast to the first type and be exemplified by any case in which there is no actual loss but in which the apparent loss represents material that as an anlage or as a portion of an

anlage was developmentally never produced at all, such as defective endosperm in maize (MANGELSDORF 1926; see especially fig. 59). This second main type of localized dwarfism is an agenesis, and likewise comprises at least two distinct kinds. There is first the type in which the process in the mutant defect shows the same time course but stops abruptly at some point in the reaction which goes to asymptotic completion in the wild type or normal. This is the type of true arrest. In the second kind of agenesis the defective process goes to completion but has a lower entirely different asymptote than the normal, as in the case of defective endosperm mentioned above.

The data on bar-eyed mosaics clearly do not favor the view that in bar an anlage for a full eye is produced and then partially destroyed by autolysis to give bar eye, but rather that in bar the materials are never produced. The localized dwarfism of bar eye is not a loss by some form of atrophy or autolysis as in vestigial but only an apparent loss brought about by agenesis, as the following argument is intended to show.

If in *BMn/w* females an anlage for full eye is produced it will necessarily, on the basis of the given genetic constitution, be also one for the production of red facets. Now if because of the presence of bar a part of the anlage is lost by autolysis the eye will be smaller. It will show the bar effect. In such females when somatic crossing over occurs, leaving a genetic constitution for full eye, less of the original anlage would be destroyed and so, on this view, the eye would be larger; and the earlier the change in genetic constitution, the larger still would be the resulting mosaic eye. But these mosaic eyes are not only larger in size, they also possess a larger white spot. So far as the white facets are concerned, we require a process of some sort which will give more and more white facets, and not merely a process which destroys less and less material for the production of red facets. A process which destroys less and less material for the production of red facets although leaving a larger total eye than that found in bar will not produce more and more white facets. Bluntly and briefly, more and more white facets cannot result from a process which merely destroys less and less material for red facets. If this general argument is justified then it is valid to draw the inference that in bar eye we are dealing with a localized dwarfism that is not an atrophy but an agenesis. There is an agenesis of the facets which are lacking in bar but which are present in the wild type. Furthermore, the analysis (see HERSH 1934b) of DRIVER's data on ultra-bar shows that, at least for the reaction during the temperature effective period, the agenesis is very likely not a true arrest as defined above but a process with a different asymptote than that found in the wild type (but see GOLDSCHMIDT 1938, p. 76 and p. 216).

What a part becomes, according to the well-known formula, is a function of its size and of its position in the whole at the time of the embryonic

determination of the part. The data on mosaics support the view that the bar factors act by altering the growth, presumably by reducing the number of cell divisions in the optic disk. After the loss of the bar factor as the result of crossing over in a *fBMn/w* fly the cell with wild type constitution divides more rapidly because of the absence of bar, and after somatic crossing over in a *fBMn/wBB* fly the addition of a bar factor reduces somewhat further the number of cell divisions in the optic disk, but not enough to shift the curve of variability of the *fBMn/wBB* flies very far toward homozygous ultrabar.

#### SUMMARY

Data on white bar-eyed mosaics of *Drosophila* recovered at 20°, 25°, and 30° in *fBMn/w* flies and in *fBMn/wBB* flies at 25°, conform as an approximation to the power function,  $y = bx^k$ . ( $y$  is the number of white facets,  $x$ , the total facet number in the mosaic eye,  $b$  and  $k$  are constants.) The values of  $k$  for the 4 sets of mosaics in the order mentioned are 3.86, 3.53, 1.76 and 2.81. As the order of eye size in any set increases the value of the constant  $b$  decreases. The presence of Minute- $n$  increases from 4 to 10 times the rate of mosaic formation over that found in the corresponding non-*Mn* flies of the same experiments. The data favor the view that bar eye considered as a localized dwarfism is the result of a process of agenesis and not one of atrophy or autolysis as in the vestigial alleles. Although the time relations are unknown the inference can be drawn from the mathematical relations that in *fBMn/w* mosaics there is a time during development which is the latest time for the change at the bar locus to wild type to result in an all-white eye. Up to this time the action of the bar factor is to depress the eye size by nearly 300 facets.

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# THE INDUCTION OF DOMINANT AND RECESSIVE LETHALS BY RADIATION IN HABROBRACON<sup>1</sup>

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Received June 15, 1938

## INTRODUCTION

GENETIC literature includes much discussion of lethal factors. Texts treat the subjects of sex-linked and autosomal lethals, lethals linked with visibles, balanced lethals and semilethals. There are many studies of induction of lethals by X-radiation and other physical agents including correlation of number of lethals with dosage given. Almost without exception the lethals referred to are recessive, transmissible from one generation to another through the heterozygote.

Dominant lethals, not transmissible since zygotes receiving them die, and evident only in their mass effect, have been discussed but little. In organisms with the ordinary type of reproduction dominant lethals would be difficult to distinguish from direct inactivation of germ cells although MULLER (1927) attributed the partial sterility of X-rayed males in *Drosophila* to dominant lethals, pointing out that although they could not be detected individually "their number was so great that through egg counts and effects on sex ratio evidence could be obtained of them *en masse*. It was found that their numbers are of the same order of magnitude as those of recessive lethals."

In the parasitic wasp *Habrobracon*, in contrast to such organisms as *Drosophila*, the occurrence of dominant lethals in the sperm can be readily distinguished from direct killing of the male gametes, as explained below. For this reason the experiments reviewed and reported in the present paper were undertaken and the results indicate that the induction of dominant lethals by radiations can scarcely be doubted.

For an understanding of the exceptional method of reproduction and heredity in this insect the following facts should be borne in mind.

Females are diploid and develop from fertilized eggs. Normal fertile males are haploid and develop parthenogenetically from unfertilized eggs.

<sup>1</sup> The work reported in this paper has been greatly aided by grants from the Committee on Effects of Radiation on Living Organisms (National Research Council). Most of the recent experiments have been carried out at the University of Pennsylvania, with the breeding work done at the Zoological Laboratory, the cold rooms for storage supplied by the Department of Anatomy at the Medical School and the X-ray treatments given by Dr. Raymond Zirkle at the Department of Radiology of the University Hospital. During the summers the breeding work has been done at the Marine Biological Laboratory, Woods Hole, Mass., and the X-ray treatments have been given there.

Biparental sterile (or near-sterile) males are diploid, developing, like females, from fertilized eggs. The haploid males have been called uniparental and azygous in contrast to the biparental zygous males and females.

Unmated females produce azygous sons only. Mated females also produce azygous sons from unfertilized eggs but in smaller numbers than unmated females and zygous biparental offspring from fertilized eggs. Whether the biparental offspring shall be daughters only or both sons and daughters depends upon the relationship of the male used in the mating. If the male is from a stock unrelated to the female, all the biparental offspring are daughters but if the male comes from a related stock, biparental sons as well as daughters are produced.

In general the azygous and biparental offspring from a mated female occur in the same ratio from the different vials through which the female is transferred (a, b, c, d, etc.) until her supply of sperm is exhausted. Subsequently (vials, e, f, etc.) azygous sons only appear.

Biparental males cannot conveniently be separated from their azygous brothers unless the mother has a recessive trait and the father has the allelic dominant. Orange-eyed females crossed with wild type (black-eyed) males produce black-eyed daughters and orange-eyed azygous sons. If biparental sons are produced they may be readily separated from their azygous brothers by their black eyes.

All daughters from outcrosses appear to be similar in viability to inbred females, although the latter are in general somewhat less fecund.

Fertilized eggs may be "female-producing" or "male-producing," the latter occurring only if parents are related. "Male-producing" fertilized eggs are less likely to hatch than "female-producing." Consequently there are more "bad eggs" and fewer biparental offspring if the mating has been with a related male, since the percentage of eggs fertilized is the same whether the male is related or unrelated. Biparental male larvae are also less likely to mature than female larvae. This further reduces offspring from related parents.

Two types of male sterility may be distinguished according as the eggs are fertilized or not. If the eggs are not fertilized, the mated female breeds like an unmated female, producing a large number of azygous sons. This occurs in the case of matings with biparental males which produce diploid sperm rarely capable of fertilizing the eggs. There are also males with abortive sperm ducts or testes which may readily be mated but transmit no sperm. Recent evidence indicates that sperm may to some extent be inactivated by high dosages of X-rays, so that they are unable to penetrate the eggs.

The most striking result of X-radiation of males is, however, the pro-

duction of sterility (or partial sterility) of a second type. In this case the sperm are not inactivated but are fully capable of fertilizing the eggs. Such fertilized eggs however, do not hatch because the sperm have a dominant lethal effect. Females mated to males with this type of sterility produce azygous sons only and in numbers equal to those produced by females mated to normal males; in other words, although they produce no females, due to dominant lethal effects of the sperm which have entered the eggs, they behave like mated rather than unmated females in respect to number of azygous sons. *Habrobracon*, then, is especially well suited for separating dominant lethal male sterility from sterility due to inactivated sperm on the basis simply of numbers of azygous sons produced by females mated to the males to be tested.

As to the nature of dominant lethals it is probable that they are due to extensive chromosomal alterations rather than to changes in restricted regions or in single genes. A lethal effect from a single gamete may be called dominant in contrast to a condition which must be present in both gametes and hence recessive.

Since in *Habrobracon* an unfertilized egg provided with a single set of genes develops normally (into a male) and since the addition of a second complete set by fertilization likewise results in normal development (into a female), it might be thought that the addition of a deficient set should not be lethal to the resulting zygote. In other words, if both  $rn$  and  $2n$  may develop normally, why should not  $rn + (n - x)$  also develop normally? The explanation is doubtless to be found in genic balance. If  $n - x$  is too small to act as a recessive, containing relatively extensive deletions for example, the balance should be so disturbed that development would be prevented. Theoretically the genic set in a sperm might become so extremely deleted by X-radiation that the fertilized egg would develop as an unfertilized egg into a male. No sex intergrades have ever been found in the treated material that might be interpreted as hyperploid males or hypoploid females. The sex types surviving after X-radiation have been fully as normal as in untreated stock.

Recessive lethal (or semilethal) factors may be a cause of "bad eggs." Half of the unfertilized eggs of a female heterozygous for a recessive lethal do not hatch.

Eggs may likewise be defective in their gross morphological or non-nuclear aspects because of unfavorable cultural conditions (very low humidity) or inadequate nutriment of females (feeding with honey instead of caterpillar juice) while females of certain genetic types lay withered eggs of irregular form.

Non-hatchability may then be due to non-nuclear causes, recessive lethals, dominant lethals or "male-producing" fertilization.



Table 1 is based on part on data published by C. H. BOSTIAN (1935) who counted eggs and offspring from females of an inbred orange-eyed stock which were bred unmated, or after mating to related or to unrelated males. Eggs averaged 21 per day regardless of the type of mating. Some of the unhatchable bad eggs from unmated mothers may be so because of recessive lethals in the stock and hence there is a somewhat greater viability of offspring (of which three-fourths are females) when mothers are mated to unrelated males. Matings with related males result in many unhatchable eggs. If the difference between bad eggs from matings of related and unrelated parents be added to the biparental sons from the

TABLE 1

*Offspring per day of inbred orange-eyed females (stock 11-0) bred unmated or mated with wild type males. The average daily egg production per female is 21.*

| TYPE OF MATING   | OFFSPRING PER DAY           |                      |                    |             |
|--|-----------------------------|----------------------|--------------------|-------------|
|  | AZYGOUS.<br>ORANGE<br>MALES | BIPARENTAL.          |                    | BAD<br>EGGS |
|  |                             | WILD TYPE<br>FEMALES | WILD TYPE<br>MALES |             |
|  |                             |                      |                    |             |
| Female unmated (or mated to male unable to transmit sperm) | 14.5                        | —                    | —                  | 6.5         |
| Outcrossing; male of an unrelated stock                    | 4                           | 12                   | —                  | 5           |
| Inbreeding; male of a closely related stock                | 5                           | 5.5                  | 1.7                | 8.8         |
| Dominant lethals in all sperm                              | 5                           | —                    | —                  | 16          |

former, it appears that female-producing and male-producing combinations occur in equal numbers (5.5 of each type per day). The table also gives the expectation in case of a mating with a male having dominant lethals in all sperm. Here all fertilized eggs are unhatchable and offspring are limited to five azygous sons per day.

#### RECESSIVE LETHALS

In a discussion of azygotic segregations from heterozygous mothers (WHITING and BENKERT 1934) deviations from equality of mutant types and wild type were explained by linkage with recessive lethals, especially when the types were otherwise of normal viability. These deviations may be very great or relatively slight indicating that the lethal may be either closely or loosely linked with the mutant locus. Not infrequently also the pedigree shows that a certain female is heterozygous for a lethal since about one-half of her daughters are lethal bearing.

WHITING (1929), in a general account of X-radiation results with *Habrobracon*, presented evidence that certain daughters of treated parents

were carrying recessive lethals. Very few offspring (males) were produced if such lethal-bearing females were unmated, but if mated they were able to produce many daughters. When the number of their sons was extremely reduced, it was suggested that the lethals were either very numerous or were balanced.

MAXWELL (1935) found no reduction in fecundity of  $F_1$  females and no deviations from the 1:1:1:1 ratio of  $F_2$  males when the wild type  $P_1$  females were treated with X-ray dosages of 2,500 r and subsequently mated to untreated cantaloup gynoid males. In a second experiment treatment of the cantaloup gynoid males with the same dosage before mating with untreated wild-type females was however very effective, reducing the average number of sons per day per  $F_1$  mother from 7.06 to 4.59. Males subjected to higher dosages showed still greater reduction in these averages. The 21 control  $F_1$  females each averaged six or more sons per day, with but two exceptions, while, among the daughters of treated fathers, 31 averaged six or more while 64 averaged three or less. This bimodality indicates that about two-thirds of the daughters of treated males carried at least one lethal. Among the 105  $F_2$  fraternities from treated males there were significant deviations from equality for cantaloup in four and for gynoid in four. MAXWELL's data suggest that recessive lethals are more readily induced in the sperm than in the eggs.

#### DOMINANT LETHALS INDUCED BY TREATMENT OF MALES

MULLER (1927) attributed the partial sterility of X-rayed males in *Drosophila* to dominant lethals. WHITING (1929) showed that in *Habrobracon* males were active and apparently uninjured after a dosage that was seven times as great as was required to induce complete sterility. With treatments below this sterilizing dosage, there was a decrease in percentage of daughters among the progeny with increasing dosages when the males were mated immediately after treatment, but there was a partial recovery of fertility as indicated by increasing percentage of females from subsequent matings on successive days.

STANCATI (1932) demonstrated dominant lethals in sperm of wild type males after treatment (2,500 r). The males were mated to females of a related stock with orange eyes, so that biparental sons were produced and could be detected. STANCATI reported numbers of offspring per vial and percentages of biparentals, including the biparental sons with the daughters. Not all matings were observed but the controls included "practically no unisexual fraternities." Among the treated, however, there were many unisexual fraternities, indicating sterilization of the males. From his figures it may be calculated that there were among the controls, 3.39 azygous sons per vial. This was increased to 4.66 among the treated

bisexual and to 4.94 among the treated unisexual fraternities. This increase might suggest inactivation of sperm, but this seems unlikely in view of later results and the low dosage. Some fraternities from unmated females may have been included among the unisexual treated. The biparental offspring, sons and daughters, are 6.25 per vial from the controls and 1.50 from the treated bisexual. This decrease is not compensated by the increase in azygous sons and hence the partial sterility of the fathers was in part at least due to dominant lethals.

WHITING (1936) showed that dominant lethals were induced in the sperm by neutrons. Treated males were mated to untreated unrelated females. While daughters decreased steadily with increasing dosages, there was no compensating increase in number of sons. Thus the sperm were not inactivated by the neutrons but were able to penetrate the eggs and prevent their development.

WHITING (1937 a and b) likewise showed a progressive increase in partial sterility due to dominant lethals when X-rayed males were mated to unrelated females. Table 2 (reprinted from WHITING 1937a) gives the data. Irregular fluctuation of sons per day may be noted. A slight increase in the 75,000 r group suggests possible sperm inactivation, but if this is significant it requires a far higher dosage than is necessary for complete sterilization by induction of at least one dominant lethal in every sperm.

TABLE 2

*Offspring produced in a given number of days by untreated orange-eyed females (stock 11-0) mated to wild type unrelated males, controls and X-rayed with various dosages.*

*(From "The Collecting Net," Woods Hole, August 7, 1937.)*

| TREATMENT<br>OF MALES | TOTAL<br>EGG-LAYING<br>DAYS | OFFSPRING |       | ♂ ♂<br>PER DAY | + ♀ ♀<br>PER DAY | SURVIVAL<br>RATE |
|-----------------------|-----------------------------|-----------|-------|----------------|------------------|------------------|
|                       |                             | ♂ ♂       | + ♀ ♀ |                |                  |                  |
| Controls              | 379                         | 459       | 1310  | 1.21           | 3.30             | 1.00             |
| 2,500 r               | 253                         | 337       | 562   | 1.33           | 2.22             | .67              |
| 5,000 r               | 309                         | 419       | 191   | 1.36           | .62              | .19              |
| 7,500 r               | 361                         | 627       | 57    | 1.74           | .16              | .05              |
| 10,000 r              | 72                          | 62        | 5     | .86            | .07              | .02              |
| 20,000 r              | 99                          | 95        | 0     | .96            | 0                |                  |
| 40,000 r              | 117                         | 157       | 0     | 1.34           | 0                |                  |
| 75,000 r              | 515                         | 890       | 0     | 1.73           | 0                |                  |
| Unmated ♀ ♀           | 675                         | 3191      | 0     | 4.73           | 0                |                  |

BISHOP (1937) showed by counting eggs and recording progeny that X-rayed males had fewer daughters than untreated and that there was a corresponding increase in bad eggs. Matings were between both unrelated and related parents. In the latter case biparental sons occurred but were not numerous enough to indicate decrease due to treatment.

WHITING (1938) showed that there was progressive decrease in biparentals, both sons and daughters, with increasing X-ray dosages when the treated males (wild type) were crossed with related females (orange-eyed). There was no compensating increase in number of azygous sons but this remained low as among the offspring of the mated controls.

For the four groups: controls, 2,500 r treatment, 5,000 r and 7,500 r, the daughters per day were respectively 1.83, 0.97, 0.31 and 0.06 and the biparental sons per day were 1.20, 0.59, 0.17 and 0.05. Fraternities from crosses of these stocks give a very high ratio of biparental sons, but nevertheless they fail to equal the daughters. It is of interest to note that decrease in daughters and in biparental sons is at the same rate, a fact that is taken to indicate that the radiation is no more lethal to one than to the other sex among the biparentals and that the type of fertilization (male-producing vs. female-producing) is not modified.

MAXWELL (1938) mated the same males on successive days to unrelated females. Dosages of 41,000 r and 142,000+r were administered to the two groups of treated males so that no daughters were produced by the treated. Sons per day averaged 1.05 for the controls, 1.41 for the lower treatment and 2.01 for the higher. Maxwell concludes that "Inactivation of sperm following higher dosages rather than partial exhaustion of sperm supply due to partial inhibition of spermatogenesis is indicated by the fact that there is no increase in males per day from the later matings."

#### DOMINANT LETHALS INDUCED BY TREATMENT OF FEMALES

Treatment of females by X-radiation was made by WHITING (1929). Mature eggs were shown to be less susceptible, while earlier stages were readily affected. Thus females fed on caterpillars and in actively laying condition had a postponed sterility since they produced offspring immediately after treatment (in vials *a*), but proved sterile thereafter. Honey-fed females, having no mature eggs, produced no offspring after equivalent dosages. In certain cases the postponed sterility was temporary for a few offspring might be produced in later life. Inviably pupae and larvae were sometimes observed after recovery, but data are not sufficient to prove that such were more numerous than usual.

In an experiment with low intensity but prolonged treatment of mated females (WHITING 1929), the percentage of daughters was decreased with increasing dosages, being 24.0—, 16.9— and 11.7— in the bisexual fraternities. No untreated controls were run. NEITA C. BOSTIAN (1931) X-rayed (3,200 to 6,400 r) inbred females mated to males of the same stock (no. 1). Offspring per female and percentage of daughters (vials *a* only) decreased with increasing dosages. When unmated females were treated (3,267 r) and subsequently mated to untreated males, offspring

per female and percentage of daughters were both higher than among the mated females treated with the lowest dosage. WHITING (1935), reporting experiments of JANE MAXWELL, ANNA R. WHITING and KATHRYN G. SPEICHER, showed that among the offspring of mated females X-rayed with dosages ranging from 3,500 to 7,000 r, the percentage of daughters decreased (38% to 7%) with increasing dosages. GREB (1933) showed that among bisexual fraternities from X-rayed mated females the average number of sons per mother was not significantly affected by a light dosage (2,000 r), although the daughters were much decreased. These experiments

TABLE 3. EXPERIMENT II/3/37/TST

*Offspring produced in a given number of days by orange-eyed females (stock II-o), (controls and X-rayed with various dosages), which were bred unmated or were mated to unrelated untreated wild type males (stock 32) either before or after treatment.*

| MOTHERS                      | TREAT-<br>MENT | DAYS<br>FER-<br>TILE | TOTAL<br>DAYS | ♂ ♂ | ♀ ♀ | ♂ ♂ PER<br>DAY | SUR-<br>VIVAL<br>RATE | ♀ ♀ PER<br>DAY | SUR-<br>VIVAL<br>RATE | % ♀ ♀  |
|------------------------------|----------------|----------------------|---------------|-----|-----|----------------|-----------------------|----------------|-----------------------|--------|
| Unmated                      | Controls       | 143                  | 143           | 488 |     | 3.41+          | 1.00                  |                |                       |        |
|                              | 1,500 r        | 81                   | 81            | 225 |     | 2.78-          | .81+                  |                |                       |        |
|                              | 3,000 r        | 110                  | 110           | 200 |     | 1.82-          | .53+                  |                |                       |        |
|                              | 6,000 r        | 20                   | 67            | 28  |     | .42-           | .12+                  |                |                       |        |
|                              | 12,000 r       | 20                   | 103           | 22  |     | .21+           | .06+                  |                |                       |        |
| Mated                        | Controls       | 289                  | 289           | 252 | 766 | .87+           | 1.00                  | 2.65+          | 1.00                  | 75.25- |
| Mated<br>before<br>treatment | 1,500 r        | 102                  | 102           | 126 | 245 | 1.24-          | 1.42-                 | 2.40+          | .91-                  | 66.04- |
|                              | 3,000 r        | 96                   | 96            | 54  | 50  | .56+           | .65-                  | .52+           | .20-                  | 48.08- |
|                              | 6,000 r        | 12                   | 85            | 13  | 5   | .15+           | .18-                  | .06+           | .02+                  | 27.78- |
|                              | 12,000 r       | 20                   | 100           | 4   |     | .04            | .05-                  |                |                       |        |
| Mated<br>after<br>treatment  | 1,500 r        | 86                   | 86            | 49  | 112 | .57-           | .65+                  | 1.30+          | .49+                  | 69.56+ |
|                              | 3,000 r        | 139                  | 139           | 70  | 250 | .50+           | .58-                  | 1.80-          | .68-                  | 78.13- |
|                              | 6,000 r        | 16                   | 70            | 3   | 15  | .04+           | .05-                  | .21+           | .08+                  | 83.33+ |
|                              | 12,000 r       | 20                   | 106           | 3   | 20  | .03-           | .03+                  | .19-           | .07+                  | 86.06  |

suggest that dominant lethals are more readily induced in the sperm than recessive lethals in the eggs.

Two experiments have recently been completed in which females were X-rayed. I am indebted to DR. RAYMOND ZIRKLE and to the Department of Radiology, University of Pennsylvania Hospital, for cooperation in giving the X-ray treatments.

Females of an orange-eyed stock (II-o) were fed on caterpillars so that they were in active egg-laying condition. They were bred unmated or were mated to azygous males (collected from unmated mothers to avoid any chance of using biparental sterile males) of an unrelated wild type stock (no. 32). Thus, no biparental sons should be produced and percentage of

daughters should be relatively high. Females were subjected to X-radiation and matings were made either immediately before or immediately after treatment. Except for the controls, eggs were therefore treated in all cases, but sperm were X-rayed only when matings preceded treatments. Data are summarized separately (tables 3 and 4) since cultural conditions were much better in the second experiment resulting in larger numbers.

TABLE 4. EXPERIMENT 2/38/GS

*Offspring produced in a given number of days by orange-eyed females (stock 11-0), (controls and X-rayed with various dosages), which were bred unmated or were mated to unrelated untreated wild type males (stock 32) either before or after treatment.*

| MOTHERS                      | TREAT-<br>MENT | DAYS<br>FER-<br>TILE | TOTAL<br>DAYS | ♂ ♂ ♂ | + ♀ ♀ | ♂ ♂ PER<br>DAY | SUR-<br>VIVAL<br>RATE | ♀ ♀ PER<br>DAY | SUR-<br>VIVAL<br>RATE | % ♀ ♀  |
|------------------------------|----------------|----------------------|---------------|-------|-------|----------------|-----------------------|----------------|-----------------------|--------|
| Unmated                      | Controls       | 225                  | 225           | 1291  |       | 5.74-          | 1.00                  |                |                       |        |
|                              | 1,500 r        | 86                   | 86            | 382   |       | 4.44+          | .90+                  |                |                       |        |
|                              | 3,000 r        | 36                   | 36            | 72    |       | 2.00           | .41-                  |                |                       |        |
|                              | 4,000 r        | 151                  | 151           | 191   |       | 1.26+          | .26-                  |                |                       |        |
|                              | 5,000 r        | 88                   | 88            | 109   |       | 1.24-          | .25+                  |                |                       |        |
|                              | 6,000 r        | 52                   | 118           | 51    |       | .43+           | .09-                  |                |                       |        |
|                              | 12,000 r       | 20                   | 135           | 28    |       | .21-           | .04+                  |                |                       |        |
|                              | 18,000 r       | 16                   | 57            | 4     |       | .07+           | .01+                  |                |                       |        |
| Mated                        | Controls       | 209                  | 209           | 292   | 953   | 1.40-          | 1.00                  | 4.56-          | 1.00                  | 76.55- |
| Mated<br>before<br>treatment | 1,500 r        | 114                  | 114           | 152   | 399   | 1.33+          | .95+                  | 3.50           | .77+                  | 72.05+ |
|                              | 3,000 r        | 124                  | 124           | 154   | 345   | 1.24+          | .89-                  | 2.78+          | .61-                  | 69.14- |
|                              | 4,000 r        | 123                  | 123           | 55    | 70    | .45-           | .32+                  | .57-           | .12+                  | 56.00  |
|                              | 5,000 r        | 208                  | 208           | 83    | 77    | .40+           | .29-                  | .37+           | .08+                  | 48.13- |
|                              | 6,000 r        | 44                   | 80            | 35    | 10    | .44-           | .37+                  | .13-           | .03-                  | 22.22+ |
|                              | 12,000 r       | 44                   | 172           | 3     | 1     | .02-           | .01+                  | .01-           | .00+                  | 25.00  |
|                              | 18,000 r       | 20                   | 103           | 11    |       | .11-           | .08-                  |                |                       |        |
| Mated<br>after<br>treatment  | 1,500 r        | 127                  | 127           | 122   | 524   | .96+           | .69-                  | 4.13-          | .90+                  | 81.12- |
|                              | 3,000 r        | 119                  | 119           | 161   | 510   | 1.35+          | .97-                  | 4.29-          | .94-                  | 76.01- |
|                              | 4,000 r        | 186                  | 186           | 106   | 434   | .57-           | .41-                  | 2.33+          | .51+                  | 80.37  |
|                              | 5,000 r        | 150                  | 150           | 108   | 266   | .72            | .52-                  | 1.77+          | .39-                  | 71.12+ |
|                              | 6,000 r        | 80                   | 97            | 22    | 91    | .23-           | .16+                  | .94-           | .21-                  | 80.53+ |
|                              | 12,000 r       | 28                   | 212           | 18    | 19    | .09-           | .06+                  | .09-           | .02-                  | 51.35+ |
|                              | 18,000 r       | 20                   | 103           | 8     | 5     | .08-           | .06-                  | .05-           | .01+                  | 38.46+ |

If there is no inactivation of sperm resulting from the treatment, the survival rate of sons per day (sons per day for the various treated groups divided by sons per day for the controls) (column 8, tables 3 and 4) should be the same for equal dosages in both experiments and whether mothers are unmated or mated before or after treatment. For the unmated series in both experiments where numbers of azygous sons are relatively high the rates are roughly parallel, but there is wide fluctuation in the four

mated series. In this connection it should be recalled that estimates of errors based merely on the numbers of offspring may be too small. A recessive lethal or semilethal occurring in a very few mothers may have considerable influence on averages of azygous sons. This is but one of the factors that may cause wider fluctuations than might be expected from the numbers appearing in the tables. Nevertheless it may be noted that in both experiments the groups in which sperm were treated have in general a higher rate of production of azygous sons. That this indicates sperm inactivation is doubtful in view of MAXWELL'S (1938) findings, but further tests would be of interest.

The decrease in azygous sons (as also in daughters) with increasing dosages is very marked at 6,000 r. At about this point complete sterility usually appears after vials *a*. The drop in average offspring per day is then due to the fact that the mother may live for many days subsequently without producing offspring. This postponed sterility may cause much fluctuation in averages if the length of life subsequent to the brief fertile period be greatly extended even in a single female. The "days fertile" (from time set until no more offspring were produced) are therefore lower (tables 3 and 4, column 3) for the females treated with higher dosages. In several of the 6,000 r groups (especially in experiment 2/38/GS, table 4) there were offspring in vials *a* but none in *b* followed by a few and sometimes several in *c* or *d*. This postponed temporary sterility suggests greater susceptibility of young oöcytes than of oögonia. Mature eggs seem to be but little affected and even after 18,000 r a few offspring may be produced.

Decrease in daughters per day is much more striking if matings are made before the treatment so that sperm are irradiated. Practically all daughters are eliminated by 12,000 r treatment of the sperm in the mated females. This is in agreement with the results from a closely similar treatment of the males (10,000 r) (WHITING 1937, see table 2 in this paper).

A few daughters are produced after 18,000 r treatment of eggs fertilized by untreated sperm.

The percentage of daughters drops rapidly when eggs and sperm are both treated, but if eggs alone are treated it is doubtful if there is any significant effect. The total number of offspring is very much reduced with higher dosages so that the apparent drop in percentage of daughters after treatment with 12,000 r and 18,000 r before mating (table 3) may be only a fluctuation. It was expected that a slight increase in percentage of daughters might appear on account of recessive lethals induced in the eggs. Were it possible to cause recessive lethals in all eggs escaping dominant lethal effects, the percentage of daughters might be increased up to one hundred. Further work may indicate whether this can be done, or whether eggs may be rendered unfertilizable but still able to develop normally into

males. Dominant lethals induced in the eggs should have no effect on the sex ratio.

#### SUMMARY

1. Dominant lethals may be induced in the sperm of *Habrobracon* by X-radiation of the males. At 10,000 to 20,000 r units all sperm have at least one lethal. With very high dosages, 41,000 to 142,000 r, some sperm are directly inactivated while many still remain active and able to carry dominant lethals into the eggs. Reduction of biparental sons and of daughters takes place at the same rate.

2. Recessive lethal-bearing daughters of treated males were twice as numerous as non-lethal bearing. Linkage with segregating visible mutants is sometimes indicated.

3. Treatment of females causes reduction in offspring per day, due either to the induction of dominant lethals or to direct killing of eggs. No change in sex ratio would be caused by dominant lethals in the egg.

4. If females are treated and subsequently mated, there is no appreciable reduction in male ratio, indicating that few, if any recessive lethals are induced in the egg.

5. Treatment of mated females causes a radical lowering of female ratio indicating that more dominant lethals are induced in the sperm than recessive lethals in the egg.

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# THE DEVELOPMENT OF TWO TAILLESS MUTANTS IN THE HOUSE MOUSE

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Received June 30, 1938

## INTRODUCTION

THE events that take place in the development of the mammalian embryo have not been subjected to an extensive causal analysis so far. The reasons for this are to be found mainly in the lack of suitable methods. It is not possible yet to use transplantation, isolation or vital staining techniques on mammalian embryos as they have been used on amphibian embryos. In the course of time it probably will be possible to analyze the mammalian embryo by transplantation and isolation just as thoroughly as has been done with the amphibian. For the present, however, the experimenter is not able "to take an active part in the course of events that take place during the embryogeny of the mammalian embryo," nor "to alter the course of events at a chosen point in a chosen manner and draw conclusions on their relations from the resulting changes." (SPERMANN 1936.)

A mutation that causes a certain malformation as the result of a developmental disturbance carries out an "experiment" in the embryo by interfering with the normal development at a certain point. By studying the details of the disturbed development it may be possible to learn something about the results of the "experiment" carried out by the gene. However to discover anything about the nature of the action of the gene is a much harder task. It is necessary for this purpose to be able to trace back all the results of the action to certain original causes. While the experimental embryologist carries out a certain experiment and then studies its results, the developmental geneticist first has to study the course of the development (that is, the results of the developmental disturbance) and can then sometimes draw conclusions on the nature of the "experiment" carried out by the gene.

In amphibian embryology the experimental analysis has led to an understanding of the causal relations of the normal developmental events. It is possible that the study of hereditary developmental disturbances and their causes in the mammalian embryo may contribute to knowledge of the causal morphology of mammalian embryology.

This paper restricts itself to the description of the development of an

<sup>1</sup> This research was aided by a grant from the Josiah Macy Jr. Foundation.

hereditary malformation which consists in taillessness in adult mice. So far it has not been possible to associate this malformation with a specific original defect, although a possible mode of origin is suggested and further investigations are being made along this line.

The genetics of the two strains of mice in which taillessness is produced (strains A and 29) has been described in outline in two papers (CHESLEY and DUNN 1936; DUNN 1937).

Taillessness in these strains is due to the interaction of the dominant gene for Brachyury  $T$  and a recessive allele  $t^0$  in the A line,  $t^1$  in the 29 line. The tailless strains breed true because of a balanced lethal condition. The homozygotes  $TT$  die at the eleventh day after fertilization (CHESLEY 1935), the  $t^0t^0$  homozygotes die shortly after implantation, and the  $t^1t^1$  homozygotes either before or just at implantation. Only the heterozygotes  $Tt^0$  and  $Tt^1$  survive to become tailless adults.

CHESLEY (1933, 1935) described the development of the short-tailed mouse, the heterozygote  $T+$ , and that of the lethal homozygote  $TT$ . It is proposed in this paper to describe the development of the tailless mouse in the A and 29 strains.

I wish to express my thanks to DR. L. C. DUNN for the suggestion of the problem and his help and criticism during the course of the investigation.

#### MATERIAL AND METHODS

The material used for the investigation of the development of the tailless mouse was obtained from matings of tailless by tailless ( $Tt^0 \times Tt^0$  or  $Tt^1 \times Tt^1$ ) and of Brachy by tailless ( $T+ \times Tt^0$  or  $T+ \times Tt^1$ ) or Brachy by heterozygous normal tailed mice ( $T+ \times +t^0$  or  $T+ \times +t^1$ ). The embryos were timed by the vaginal plug method. Male and females were left together for approximately fifteen to twenty hours, after which time the female was examined for a vaginal plug. Thus the timing of the embryos was accurate within  $\pm 10$  hours. Since there is a high degree of variability in the chronological and physiological age of the embryos it was sufficient for the purposes of this investigation to time the embryos within  $\pm 10$  hours in order to obtain a complete series of tailless embryos.

The embryos were obtained from the mother in the following way. The mother was killed with ether and the uterus with the ovaries was taken out and immersed in a .75 percent saline solution. The number of embryos, their position in the uterus, and any distinct size difference observable among the embryonic capsules were recorded. Up to the ninth day of development some of the litters were dissected out of their capsules, closely examined and recorded and then preserved for imbedding and sectioning. Since in these early stages of development the embryos are easily injured when dissected out of their protecting membranes, some of

the litters were preserved and sectioned in their capsules. From the tenth day of development on the embryos were always dissected out of their capsules for recording and preservation.

All embryos were fixed in Bouin's fluid. They were imbedded in paraffin and serial sections were made at  $6\mu$  to  $15\mu$ , depending on the size of the embryo. The sections were stained in Delafield's Haematoxylin and counterstained in eosin.

For the purpose of studying the development of the tailless mouse in line 29, 21 litters from matings of tailless by tailless, Brachy by tailless and heterozygous normals by Brachy or tailless were dissected out. A total of 155 embryos was obtained. Fourteen litters yielding 100 embryos were ten days or older. At this age future taillessness can be diagnosed: of these 100 embryos 27 were tailless. In litters younger than ten days (7 with a total of 55 embryos) future taillessness could not be diagnosed. In the A line 23 litters consisting of 202 embryos were dissected out for the study of the development of the tailless mouse. Of these 18 could be diagnosed to be tailless by gross and histological examination. Thirty-three more embryos of the total were known to be genetically tailless because they came from matings of tailless by tailless. In 13 litters yielding 105 embryos, which were younger than 10 days and resulted from Brachy by tailless or Brachy by heterozygous normals, future normal and tailless mice could not be distinguished from each other. For embryos older than 10 days, normal litter mates were used as controls. Besides these, ten normal litters were dissected out, yielding a total of 75 normal embryos which were also used as controls. Development proceeds identically in the tailless embryos of the 29 line and in those of the A line. The following description was first made for the 29 line and was then proved to be true also for the A line.

#### DESCRIPTION OF THE ADULT TAILLESS MOUSE

The morphology of the adult tailless mouse has been thoroughly described by KOBOZIEFF (1935). By means of a great many radiographic pictures he demonstrated the high degree of variability in the malformations of the sacral and caudal regions of the spine of the tailless mice. The adult tailless mouse in some cases does not possess the slightest trace of a tail, but in most of the cases there is a short filament present, consisting of skin and connective tissue only and varying in length from 2 to 10 mm. Malformations in the spine consist of fusions of lumbar vertebrae with each other or with the sacrum, or of simultaneous fusions of both lumbar vertebrae inter se and with the sacrum, or of fusions of the entire lumbar region with the sacrum, and of fusions in the caudal region. Furthermore there is found a failure of ossification in the spine and incomplete fusion

in the middle line of the vertebrae. The number of vertebrae in the lumbar and sacral regions of the tailless mice is constant in the majority of cases, five in the lumbar and four in the sacral region. However a small degree of variability has been found in other cases, the number of vertebrae in the regions mentioned above being augmented or reduced. There is a high amount of variability in the manner of termination of the spine. It may terminate in the tailless mouse anywhere between the first sacral vertebra and the fourth caudal. Sometimes the spine ends quite abruptly as if it were cut off at the level of the intervertebral disc. Most often, however, the spine seems amputated not at the level of the intervertebral disc but across a vertebra. The last vertebra may be rounded off, it may lack all processes or it may be elongated more or less. In certain cases the termination of the spine shows a progressive decrease in the size of several vertebrae, reminding one of the pygostyle of a bird, or the os coccygis of tailless mammals. Sometimes several terminal vertebrae fuse. There are cases where the spine ends with a free fragment or with a partly luxated vertebra, or even with fragments reminding one of aberrant vertebrae originating from a more distal part of the tail.

In newborn tailless mice, according to our observations, lesions in the region of the sacrum are found frequently. In these cases blebs or haematomata are observed underneath the epidermis. These blebs represent cysts of spinal cord tissue. The spine itself terminates anterior to such a cyst and the last part of spinal cord which does not find any protecting vertebrae forms the cyst. In the adult, apparently, no trace of this lesion can be found externally. In a few extreme cases paralysis of the hind limbs has been observed in newborns, due probably to absence of nerves as a result of extreme shortening of the spinal cord. In dissection of one such case (29 line) no ventral roots were found in the last part of the spinal cord. This would account for the paralysis of the hind limbs. In two other cases (A and 29 line) two tailless mice were born without an anal opening. One of these newborns had also an abnormally small genital papilla.

#### DEVELOPMENT OF THE TAILLESS EMBRYO

For the study of the early stages of the embryogeny of the normal mouse up to the eighth day after fertilization three papers by SOBORTA (1895, 1903, 1911) have been used. An excellent Normentafel was published recently by HENNEBERG (Keibel's Normentafel 1937) for the rat, in which development is extremely similar to that of the mouse.

The development of the tailless embryo proceeds normally up to the eleventh day after fertilization. The tail bud becomes distinct on the ninth day of development. It grows and lengthens and at about the end of the tenth day the tail of the normal mouse begins to grow very fast and length-

ens considerably (fig. 1a). The tail of the prospective tailless mouse also grows and lengthens rapidly but at the beginning of the eleventh day a sharp constriction sets in at the proximal end of the tail (fig. 1b). This constriction always occurs at the same level and demarcates the tail from the trunk. While on the tenth day of development the somite number in the tail of both the normal and the tailless mouse is the same (it averages 6) there is a marked difference on the eleventh day. At this time it averages twenty in the normal mouse and fifteen in the tailless mouse. These numbers indicate that the somites in both tails have increased in number, in the tailless mouse however to a lesser degree than in the normal mouse. On the twelfth day the somite number in the tail of the normal mouse averages twenty-seven, while that of the tailless mouse averages twenty-one. From the thirteenth day on, no somites are recognizable in the tail of the tailless mouse by gross examination.

From the eleventh day on, there is a marked difference in the appearance of the tail of the normal and that of the tailless mouse. The constriction at the proximal tail end becomes more and more apparent. While in the normal mouse the trunk and the tail continue in one unbroken line, there is a sharp dividing line between the trunk and the tail in the tailless mouse (fig. 2a and b).

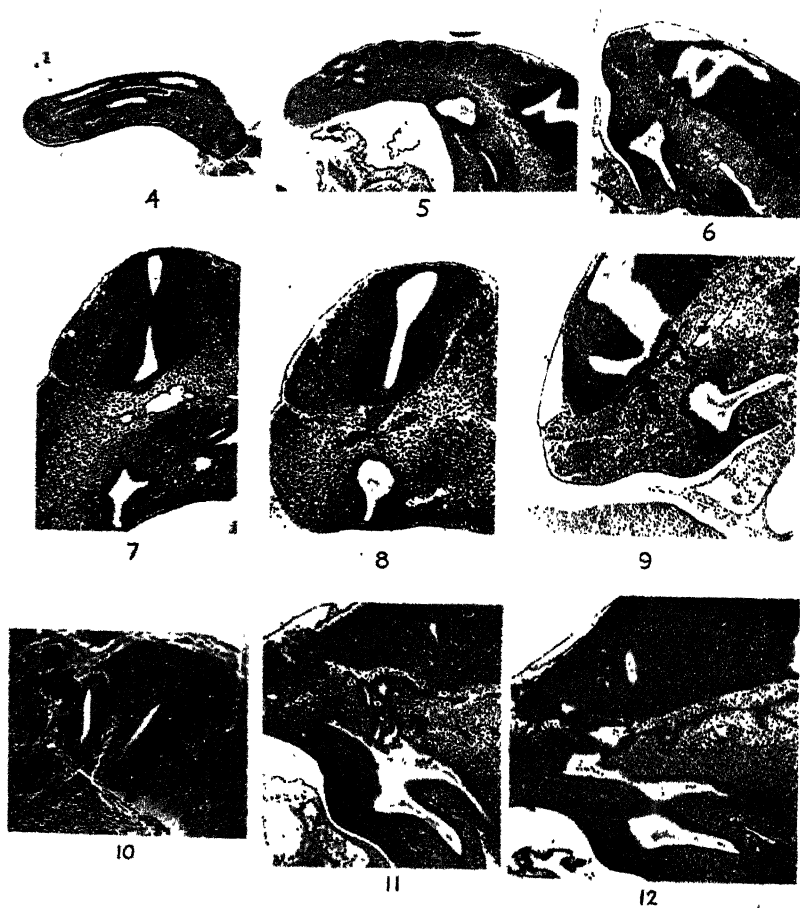
The tail of the future tailless mouse reaches its maximal length on the twelfth day of development. From then on it decreases in length and thickness, and finally, on the fifteenth day, there is but a small filament left (fig. 3b). In most cases this filament persists during the lifetime of the mouse; in a few cases, however, it is resorbed, and the mouse is born with no trace of a tail whatsoever.

Irregularities of the tip of the developing tail are quite frequently observed in both the normal and the future tailless embryo. In two litters of normal mice which were used for controls—one dissected on the 13th day after fertilization, the other on the 14th day—embryos with forked tail tips were found. Forking of the tail tip was also observed frequently in tailless embryos (fig. 1b). It occurred in both normals and abnormals most frequently at the age of 13 or 14 days, at a time when the growth rate of the tail is very high.

The gross examination clearly demonstrates that the absence of the tail in the adult mouse is not due to a complete suppression of the anlage but that the tail of the future tailless mouse develops quite normally up to a certain point after which some process sets in which effects retrogression and resorption of the tail.

#### HISTOLOGICAL EXAMINATION OF THE TAILLESS EMBRYO

The histological examination of embryos makes it possible to diagnose



FIGURES 4-12

There exists thus a striking difference between the tail bud and the elongated tail of the normal and of the mutant mouse. While in the normal mouse all structures present in the tail bud are drawn out into the elongated tail, the elongated tail of the future tailless mouse does not contain all of the structures present in the tail bud; it lacks the notochord. It seems that the notochord has reached its furthest posterior extension in the tail bud of the mutant embryo; it does not lengthen during further growth of the tail. The appearance of diverticula and ventral branches of the notochord in the posterior trunk region of the mutant (to be described below) seems to show that the notochord in its posterior part still possesses certain growth capacities although it does not grow into the elongated tail.

Apart from the tail, the most posterior part of the trunk also shows abnormalities. The structure most abnormal here is the notochord which in this region quite frequently sends off ventral branches. Figures 6 and 8 show such a ventral branch of notochord which cuts across the aorta and comes into contact with the epithelium of the cloaca. Furthermore, in the normal mouse embryo there is a wide space between the cloaca and the neural tube filled with mesenchyme (fig. 7). In the tailless mouse the space between cloaca and neural tube is very much smaller. The posterior trunk region seems to be compressed in a dorso-ventral direction (fig. 8).

Figures 11 and 12 show very abnormal conditions in the posterior trunk

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#### EXPLANATION OF FIGURES 4-12

- 4) Parasagittal section of tail of normal embryo. 3040, 3r. Age 10 days. Neural tube, notochord, hind gut.
- 5) Parasagittal section of tail of tailless embryo. 3040, 5l. Line 29. Age 10 days. Notochord entirely absent in tail. Neural tube, hind gut, somites. Last trace of notochord ventral to neural tube in posterior trunk region.
- 6) Oblique section of posterior trunk region of tailless embryo. 2624, 4r. Line 29. Age 11 days. Ventral branch of notochord in contact with epithelium of cloaca. Bleb between posterior end of neural tube and epidermis.
- 7) Oblique transverse section of posterior trunk region of normal embryo. 3040, 4l. Age 10 days. Notochord small rod ventral to neural tube.
- 8) Oblique transverse section of posterior trunk region of tailless embryo 3040, 1r. Age 10 days. Ventral branch of notochord below neural tube cuts across the aorta and comes into contact with epithelium of cloaca. Smaller space between neural tube and cloaca than in normal litter mate in figure 7.
- 9) Parasagittal section of posterior trunk region of tailless embryo 2624, 4r. Line 29. Age 11 days. (See figure 6.) Notochord running ventral to neural tube shows a number of branches and diverticula.
- 10) Parasagittal section of posterior end of neural tube of tailless embryo 2505, 1r. Line 29. Age 12 days. Neural tube bends laterally at its end. Diverticula of neural tube.
- 11) Parasagittal section of posterior trunk region of tailless embryo. 3233, 7l. Line A. Age 11 days. Fingerlike branches of hind gut that are in contact with the notochord which can be seen ventral to the neural tube.
- 12) Parasagittal section of posterior trunk region of tailless embryo 3233, 1r. Line A. Age 11 days. Notochord—ventral to neural tube—shows a number of ventral branches and is in contact with the highly malformed hind gut.

region of tailless embryos at the age of 11 days. Fingerlike branches of the hind gut establish a connection between hind gut and notochord while the notochord itself also sends off ventral branches.

In considering the abnormalities just mentioned, it should be remembered that during its development the notochord passes a stage at the age of 8 days when it is partly incorporated as chordal plate in the dorsal wall of the gut (HUBER 1918). This stage is followed by one in which the chordal plate again separates from the wall of the gut. The possibility exists that failure of the notochord to separate entirely from the gut might be responsible for the presence of the branches connecting notochord and hind gut, or notochord and cloaca.

On the eleventh and twelfth days of development the abnormalities in the tail and posterior trunk region of the tailless embryo become more and more marked. In sections of the constricted tail no notochord is found. For a short while, neural tube and gut, both not straight but curled and bent, are still to be found in the tail. Soon these structures disappear; they probably are resorbed. The last structures recognizable in the tail are the somites and blood vessels, and finally by the fourteenth day only mesenchyme and skin are left in the tail filament.

On the eleventh day the neural tube and the hind gut still extend further distally than does the notochord. The notochord in its posterior part sends off branches ventrally (fig. 9) which frequently come in contact with the cloaca. The last part of the notochord usually is not straight but bent ventrally. It may be forked at its end, and even the branches themselves sometimes are forked at the end. On the twelfth day the termination of the neural tube of the tailless embryo is found in the most distal part of the trunk. No filamentum terminale is present. The neural tube ends rather abruptly and often is not straight but curled. Frequently a bleb is found between the neural tube and the epidermis of the back (figs. 6 and 9). In the neighborhood of the termination of the neural tube small fragments of neural tube tissue are found in the surrounding mesenchyme. In embryo 2505, 11 (fig. 10) a number of diverticula of the neural tube have formed at its posterior end.

The abnormalities in the posterior region of the trunk are very variable. The notochord is always abnormal, but it may have a variable number of branches which may or may not be in contact with the epithelium of the cloaca. Frequently connections between the notochord and the cloaca are established by fingerlike branches of the hind gut (figs. 11 and 12). The space between neural tube and cloaca, containing mesenchyme, may be more or less compressed. The termination of the neural tube in this region looks different in different animals. There may be some small fragments of neural tube tissue in the region of the end of the neural tube.



Sometimes a bleb forms dorsal to the neural tube and immediately beneath the skin. The variability in the tailless embryos corresponds to that in the tailless adults. There is no strict uniformity in the abnormalities of either.

#### DISCUSSION

The development of hereditary taillessness in two strains of mice has been described. Taillessness in these strains is due to the interaction of  $T$  (Brachyury gene) with either  $t^0$  (A line) or  $t^1$  (29 line). The development of the short-tailed mouse (Brachy heterozygote  $T+$ ) has been described by CHESLEY (1935). There are a great many similarities in the development of the short-tailed and the tailless mouse. In both cases the tail is first formed normally and then later a constriction sets in, with a resulting resorption of that part of the tail and all the structures in it that lie distally to the point of constriction. In both the short-tailed and the tailless mouse the constriction appears on the eleventh day of development. The same kind of malformation of the notochord is observed in both of them, shortening of the notochord and the appearance of ventral branches in the posterior trunk region which come in contact with the gut epithelium. A difference between the heterozygous Brachy and the tailless mouse lies in the fact that in the short-tailed mouse the place varies at which the constriction sets in; the tail may become constricted anywhere between its proximal and its distal end. In the tailless mouse, however, the constriction always occurs exactly at the proximal end. Since in the adult tailless mouse the number of missing vertebrae in the sacro-caudal region varies, it follows that in the tailless embryo there must be a high degree of variability in the processes which take place in the reduction of the vertebral column. A special study of the development of the vertebral column in the tailless mouse has not yet been made. The high degree of variability in the posterior trunk region of the tailless embryo as far as the notochord is concerned has been mentioned above. However, the *place of tail constriction* is always the same in the tailless mouse.

This case of the tailless mouse reminds one also of certain wing mutants in *Drosophila* (GOLDSCHMIDT 1937). In both, development is normal up to a certain critical point. At this point in the tailless mouse, a resorption of the tail whose anlage had been perfectly normal, takes place. A degeneration of the tissue of the wing margin occurs in the mutant fly.

The present data do not give any answer to the question whether there is a time element involved in the different action of the Brachy heterozygote ( $T+$ ) and the tailless heterozygote ( $Tt$ ). One might expect at first to find that the process which produces complete taillessness sets in earlier than the process which produces a short tail. From external observations, however, it seems that the constrictions of the tail—that resulting

in taillessness as well as that leaving a short tail—set in at the same time, namely at eleven days. Since the short-tailed embryos (CHESLEY 1935) were timed within  $\pm 12$  hours and the tailless embryos were timed within  $\pm 10$  hours, it would be impossible to decide from the existing data whether a *small* time difference exists in the onset of the resorption process. Certainly no striking time difference was found. From histological observations also there is no evidence of a time difference in the onset of the two processes. CHESLEY found definite abnormalities of the notochord in heterozygous embryos at ten days, that is, before the appearance of a constriction in the tail. The same was found to be true in the tailless embryo at ten days, before any abnormalities could be detected grossly. From the data it seems as if the abnormalities in the notochord at ten days were greater in the future tailless embryo than they are in the short-tailed embryo. However no conclusive evidence of this exists, since from CHESLEY's data the exact extent of the notochordal abnormalities in the tenth day embryo is not clear.

The structures affected in the embryo in the tailless mutation are the neural tube, the notochord, the mesoderm in the tail and in the posterior trunk region, the cloaca (contact of its epithelium with the notochord). According to SOBOTTA the embryonic mesoderm in the mouse embryo arises from the wall of the primitive gut. The primitive gut of the mouse is a small blind canal about  $30\mu$  in length which runs obliquely from the cell mass of Hensen's node (at the anterior end of the primitive streak) toward the yolk sac cavity in an antero-ventral direction. It opens into the yolk sac cavity. The primitive gut in the mouse corresponds to the so-called neurenteric canal in other mammalian embryos (SOBOTTA 1911). Aside from the embryonic mesoderm, the wall of the primitive gut also gives rise to the notochord and to parts of the wall of the future gut. This seems to suggest that the malformations found in the tailless embryo might be traced back to some kind of malformation in the primitive gut region. Either some definite structure in the primitive gut has been affected by the mutation, or the primitive gut as a whole might have become affected at a certain time when it gave rise to the posterior part of the notochord, to the wall of the gut or the cloaca and to the mesoderm in the tail and posterior trunk region.

CHESLEY from his results concludes that "the abnormality of the notochord is one of the more fundamental of the disorders involved, and that the condition of the neural tube is either wholly or in part due to the abnormality of the notochord." CHESLEY further cites examples from the literature indicating the significance of the notochord for the development of the neural tube. Our data point in the same direction. The notochord

seems to be the structure first affected in the tail, malformations of the neural tube follow. While at the end of the tenth day of development the notochord of the mutant mouse differs from that of the normal by ending in the posterior trunk region and not extending into the tail, the neural tube of the mutant mouse is still normal at this time and extends into the elongated tail. Aside from this time difference in the onset of the developmental disturbances in the notochord and the neural tube, it ought to be noted that the elongated tail of the tailless mouse *never* contains any notochord, while it does contain neural tube up to the time when it becomes resorbed. Never were malformations of the neural tube found without simultaneous abnormalities in the notochord. In young stages, on the other hand, it is possible to find malformations in the notochord without associated abnormalities in the neural tube. Our data do not give *conclusive* evidence for conceiving the malformations of the neural tube as secondary to the disorders of the notochord, but they point in this direction.

If it were possible to establish definitely that abnormalities exist in the region of the primitive gut in early stages of future tailless mice, one would be justified in ascribing to these regions of the primitive gut the responsibility for the development of the malformed structures in the older embryos. At the same time one would be justified in drawing from these abnormal conditions conclusions on the causal relations of these same structures in normal development. Further work is being done in this direction.

Death of the homozygous lethal in the 29 line takes place either before or just at implantation. The homozygous lethals in the A line are found dead shortly after implantation, at the age of six days. The morphology and the development of these lethal types are being investigated.

#### SUMMARY

- 1) The development of tailless mice of two strains (line A and line 29) is described.
- 2) Embryos that are going to be tailless can be distinguished from their normal litter mates at the beginning of the 11th day after fertilization on gross examination, by a constriction that separates the tail from the trunk in the tailless embryos.
- 3) The tail which grows normally up to the 11th day retrogresses after the constriction has taken place and is then resorbed. By the 14th day only a small filament is left in place of the tail.
- 4) By histological examination it was found that the constricted tail contains somites, neural tube and hind gut, but no notochord on the 10th and 11th days after fertilization.
- 5) From the 12th day of development on, the tail filament contains no

neural tube and hind gut; soon the somites disappear also, and finally only mesenchyme is left in the filament.

6) The notochord is reduced in the posterior trunk region of the tailless embryos and sends off ventral branches. The neural tube is reduced also in the posterior trunk region and has diverticula. The space between neural tube and cloaca is smaller than in normals. No irregularities in the anterior body region were found in the tailless embryos.

7) From the present data it is not possible to conclude definitely that the notochord is the structure primarily affected in this mutation and the neural tube only secondarily. The results do, however, point in this direction.

8) The possibility that the abnormalities in the tailless mouse trace back to a malformation of the primitive gut region in an early stage of embryogeny is discussed.

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# GENETIC AND ENDOCRINE STUDIES ON A TRANSPLANTABLE CARCINOMA OF THE OVARY

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Received July 2, 1938

## INTRODUCTION

A CARCINOMA of the ovary has been maintained by successive transplantations in mice of the CBA strain for nearly three years. In the first study with this tumor, it was determined that only normal males of the CBA strain would show progressively growing tumors during a three-month period. It was also ascertained that the growing transplanted tumor secreted appreciable amounts of estrogen. The criteria of hormone secretion of the transplanted tumors were: (a) long continued estrous vaginal smears which, after removal of the tumors reverted to the diestrous type in castrate females; and (b) growth of the rudimentary mammary glands of males as the transplanted tumors grew. Insufficient hormone was secreted to affect the pelvis (STRONG, GARDNER and HILL 1937).

The above conclusions reopened the problem of immunity to this transplanted neoplasm. The transplantation of neoplastic tissue (primarily based upon carcinoma of the mammary gland) demonstrated that the progressive growth of the tumor was determined by genetic factors inherited according to the accepted laws of mendelian heredity. No functional characteristic of the grafted tumors, however, has been demonstrated in these tumors growing in genetically controlled strains of mice. The presence of multiple factors underlying susceptibility and immunity to the previously grafted tumors has complicated the physiological factors that should have been evident. In the only case in which susceptibility and immunity to a grafted tumor was apparently controlled by a single mendelian factor, the especially selected strain of mice was lost before adequate physiological proof was obtained (STRONG 1926).

The present tumor, a carcinoma of the ovary, was a suitable tissue for our purpose. The sex difference encountered in the natural susceptibility or immunity to the tumor in inbred CBA mice suggested that a simple genetic mechanism was involved. Again, the hormone factors involved in sex physiology are so well known that ample opportunity of investigating

<sup>1</sup> This experiment has been made possible by grants from the International Cancer Research Foundation, the Anna Fuller Fund and the Fluid Research Funds of Yale University, School of Medicine.

this problem in relation to the grafted tumor should not be further delayed. The secretion of estrogen by the tumor during its growth as a transplant is a type of physiology which can be easily measured. Other hormone preparations known to influence (1) follicular development of the ovary (the tumor probably arose from granulosa cells) and (2) sex physiology, should therefore be investigated. Finally the action or interplay of genetic and hormonal agencies in building up the physiological behavior of the individual seems to be indicated.

## RESULTS

The genetic factors involved in the successful growth of the grafted tumor were studied by (1) the inoculation into normal male and female mice of the CBA (STRONG 1936) strain, (2) males and females of the A strain (STRONG 1936a) and (3) the hybrids of the first two filial generations produced by crossing individuals of these two inbred strains.

### *Inoculation into normal CBA mice*

One hundred and sixty-eight female and 153 male mice of this strain were inoculated with the tumor. All males grew the tissue progressively (100 percent susceptible). Only eight of the normal females grew the tissue,

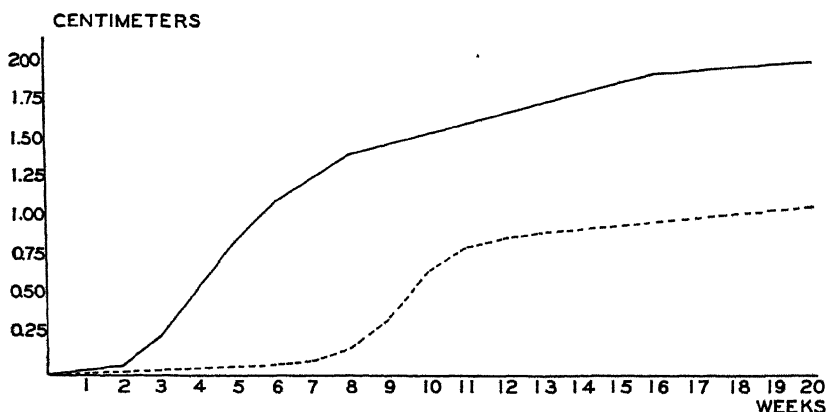


FIGURE 1.—The average growth rate of the transplant in (a) male mice (solid line) and (b) female mice (dash line) of the CBA strain. Time in weeks is plotted along the base line; size of tumor (greatest diameter in centimeters) is plotted along the vertical line.

and of these eight, seven occurred in the first two transfer generations. After a lapse of time (approximately a year) a new series of mice of the CBA strain were inoculated with the tumor and the females were kept for a considerable period after the males had all died with large tumors. Approximately eighteen percent of normal females of the new series eventually grew the tissue. In another experiment, a few female mice grew the

transplanted tissue to the size of a pea over a period of eighteen months. The sexual difference encountered was not an absolute one but rather one of degree. Whether more normal females of the CBA would later grow the tissue is of course not shown by the present data. The comparative analysis of susceptibility and resistance to the grafted tumor was significant only when the time relationships existing after the mice were actually inoculated were taken into consideration. The transplants grew much faster in males than in females (fig. 1). The tumors after a period of relatively rapid growth, were infiltrated with heavy calcified deposits, thus becoming rock-like, after which they did not increase much in size.

*Results of inoculation into  $F_1$  individuals*

Since a sex difference was encountered in the inoculation of normal individuals of the CBA strain, the outcross to the A strain was made in both directions. Seventy-nine mice produced by crossing CBA females

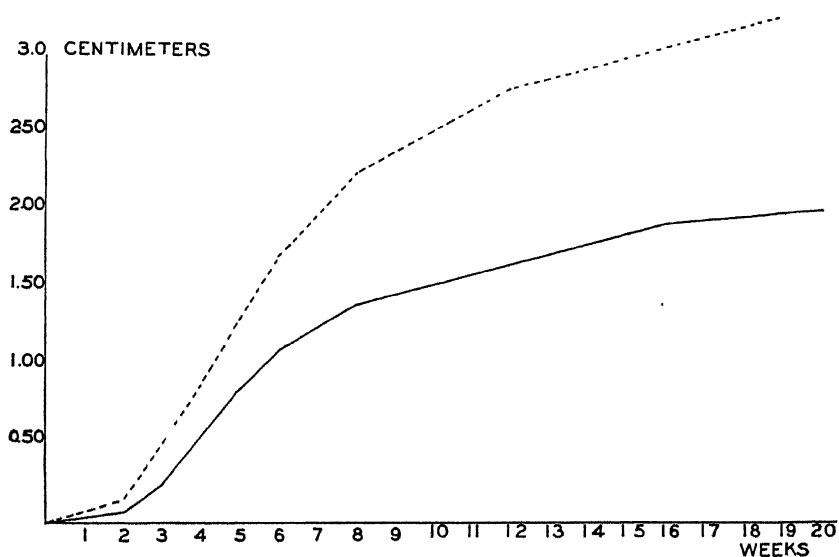


FIGURE 2.—The average growth rate of the tumor in (a) normal male mice of the CBA strain (solid line) and (b)  $F_1$  male mice obtained by a cross between the CBA and A strains. Heterosis increases the growth rate of the grafted tumor.

with A males were inoculated with the tumor; of these, 26 were males and 53 females. All of the 26 males grew the tissue progressively; whereas only 10 of the 53 females grew the tumor. The percentage of female  $F_1$  individuals growing the tissue (18.8%) was approximately the same as that obtained in purebred CBA females. In the other cross when A females were mated to CBA males, 91 mice were obtained. Of these, 44 were males and

47 females. All the males and 9 of the females grew the tumor. Of the  $F_1$  females produced by this cross 21.3 percent thus grew the tumor or approximately the same proportion as that obtained in the first  $F_1$  population and in the original CBA females. Apparently neither sex linkage nor sex limited inheritance was involved in susceptibility to this transplant. That both the males and females of the  $F_1$  generation give the same percentage of susceptible individuals would indicate, however, that susceptibility to the transplanted tissue is intrinsically determined. The evidence indicates that a non-genic sex-limited influence is probably involved.

Male mice of the  $F_1$  generation grew their tumors at a more rapid rate than male mice of the CBA strain, as indicated in figure 2. This finding has been encountered several times and indicates that heterosis is involved in the rate at which a transplanted tumor grows, as postulated by STRONG (1926a).

*Results obtained in the  $F_2$  generation*

The data obtained by inoculating the tumor into  $F_2$  individuals are presented in table 1.

|   | CBA ♀ × A ♂ |     |         | A ♀ × CBA ♂ |     |         | AVERAGE<br>PERCENT<br>PERCENT |
|---|-------------|-----|---------|-------------|-----|---------|-------------------------------|
|   | +           | —   | PERCENT | +           | —   | PERCENT |                               |
|   |             |     | +       |             |     | +       | +                             |
| ♀ | 8           | 148 | 5.1     | 16          | 196 | 7.5     | 6.5                           |
| ♂ | 20          | 80  | 20.0    | 40          | 52  | 38.4    | 29.4                          |

The data obtained in the male mice will be discussed first. It appears that multiple mendelian factors (probably four) are involved in the successful growth of this transplantable carcinoma of the ovary. The expectation for independent multiple factors in the  $F_2$  generation is given in table 2.

| TABLE 2    |         |              |
|------------|---------|--------------|
| NO FACTORS | RATIO   | PERCENT<br>+ |
| 1          | 3:1     | 75.00        |
| 2          | 9:7     | 56.25        |
| 3          | 27:37   | 42.18        |
| 4          | 81:175  | 31.64        |
| 5          | 243:781 | 23.73        |

The percentage obtained (29.4) with this  $F_2$  generation of male mice lies between that expected for four (31.64%+) or five (23.74%+) factors, nearer that expected for four factors.

The genetic analysis of susceptibility to the transplant for female mice of the same inbred strain was, however, not so clear. Definite ratios were



obtained both in pure strain individuals as well as in the outcross generations to pure strains thus indicating that intrinsic factors are involved in the process of transplantation. The same diminution of susceptibility is obtained in the  $F_2$  individual females as was seen in a similar analysis for males in the same experiment. These comparative data are shown in table 3.

TABLE 3

|             | PERCENT + |      |
|-------------|-----------|------|
|             | ♂         | ♀    |
| CBA         | 100       | 18.2 |
| $F_1$       | 100       | 20.1 |
| $F_2$       | 29.4      | 6.5  |
| $F_1 > F_2$ | 3.4       | 3.1  |

It is tentatively concluded, from the above analysis, that approximately four mendelian factors are involved in the successful transplantation of the carcinoma of the ovary in both males and females. The complete multiple-factor complex manifests itself in male mice only. Apparently some physiological mechanism within the female inhibits or prevents the full manifestation of its genetic constitution.

In order to test out this hypothetical mechanism that underlies susceptibility and resistance to the transplant an endocrinological investigation was undertaken.

#### STUDIES OF HORMONE TREATMENT ON TUMOR RESISTANCE

*Effect of castration.* Thirty-one male mice of the CBA strain, castrated at approximately six weeks of life, were inoculated with the tumor two weeks later. Of these, only fourteen grew the transplant. When the growth

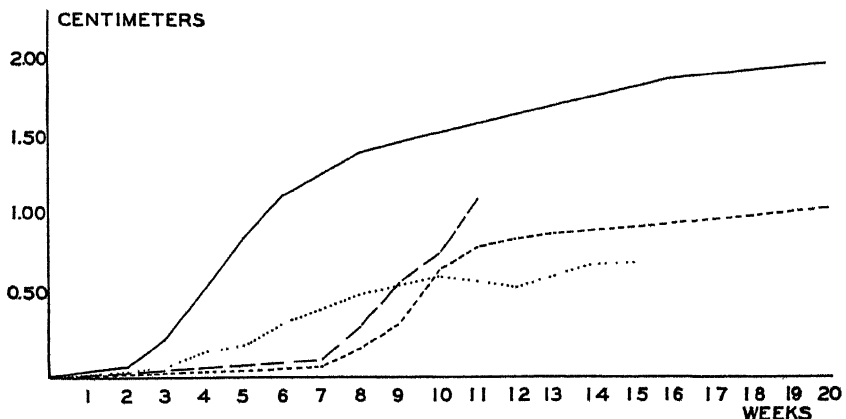


FIGURE 3.—The average growth rate of the transplant in (a) normal male mice (solid line), (b) normal female mice (long dash line), (c) castrate male mice (dotted line) and (d) male mice receiving estrogen (short dash line).

rate of the tumor is compared to that obtained in normal male and female mice of the same strain (fig. 3) it is seen that castration not only diminished incidence but also decreased the growth rate of the transplant similar to that obtained in normal female mice of the same strain.

Twelve female mice of the CBA strain were castrated at six weeks of age and inoculated with the transplant. Four of these grew the tissue progressively and slightly faster than did normal female mice of the same strain. Daily vaginal smears disclosed the presence of some estrogen but in insufficient amounts to keep the castrate female in continuous estrous.

*Hormone injections.* Since the incidence and growth rate of this transplanted ovarian carcinoma was related to the presence of the gonads, especially the testes, it might be affected by the sex hormones or gonadotropic hormones. This hypothesis was tested by injection of various hormones into 115 CBA mice in which the tumor had been transplanted under the skin in the region of the right axilla. Sixty females and 55 males were used, half of the females and 25 of the males were castrated. Five castrate and five non-castrate mice of each sex served as controls for the similar group which received daily injections respectively, of 25 i.u. of estrogen-2, (Folliculin Benzoate or Progynon B<sub>2</sub>),<sup>2</sup>  $\frac{1}{4}$  mg. Progesterin (Prolutin and Progesterin  $\frac{1}{2}$  r.u. follicle stimulating hormone (Prephysin), 2.5 r.u. pregnant mare's serum (or Gonadin serum) and 0.05 cc. of egg albumen. The 0.05 cc. egg albumen was used as a control for the protein in the gonadotropic hormones. As Progesterin had only a slight inhibitory effect on the growth rate of the tumor, it was used only in the normal male and female series, and 200 gamma (weekly) of male hormone (testosterone propionate-9) was used in the castrates.

## RESULTS

The growth rate of the transplants in male mice receiving pregnant mare's serum was not affected (fig. 4). On the contrary, female mice receiving pregnant mare's serum grew their transplants at a more rapid rate than their controls (fig. 5). This enhanced growth rate may be due to (1) a "mutation" of the tumor similar to those encountered by STRONG

<sup>2</sup> 1. The Folliculin Benzoate was obtained from the British Drug House, London, England, and Progynon B was supplied through the courtesy of Dr. E. Schwenk of Scherring Corporation.

2. The Prolutin used was obtained through the courtesy of Dr. E. Schwenk of Scherring Corporation, and the Progesterin through the courtesy of Dr. O. Kamm of the Parke-Davis Company.

3. The follicle stimulating hormone, Prephysin, was obtained from Chappel Bros., Inc., Rockford, Illinois.

4. The pregnant mare's serum was supplied by the Parke-Davis Company, through the courtesy of Dr. O. Kamm and the Gonadin was purchased from Cutter Laboratories, Berkeley, California.

5. Testosterone Propionate, "oreton," was supplied through the courtesy of Dr. E. Schwenk of the Scherring Corporation.

(1924, 1926b), BITTNER (1930, 1931) and CLOUDMAN (1932) with transplantable carcinomata of the mammary gland, or (2) the stimulation of the growth rate indirectly or directly without having undergone any cellular

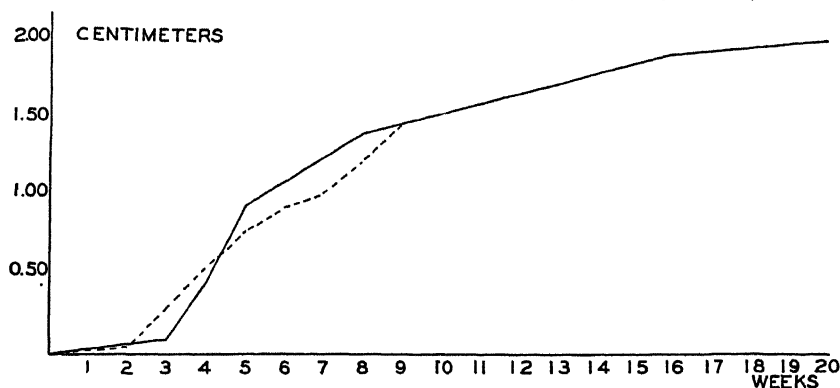


FIGURE 4.—The average growth rate of the tumor in (a) male mice (solid line) and (b) male mice receiving pregnant mare's serum (dash line). There is no difference between the two curves.

change. In order to test these two hypotheses, subsequent grafts were made from these enhanced growing tumors. The growth rate of these was the same as that obtained with the original tumor in male mice of the CBA strain. The second conclusion, therefore, seems to be the more logical one. In addition to a very rapidly growing tumor, one of the mice receiving pregnant mare serum also had several metastatic nodules in the lungs. This

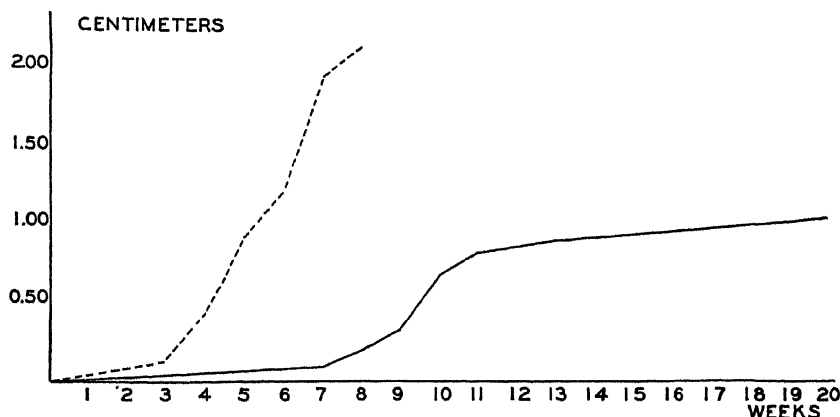


FIGURE 5.—The average growth rate of the transplant in (a) female mice (solid line) and (b) female mice receiving mare's serum (dash line). Female mice receiving pregnant mare's serum grow their tumors at an enhanced rate.

is the only mouse of several hundred examined that had a metastasis from this tumor. The tissue from the lung metastasis has been carried on by subsequent transplantations for four generations without any obvious

deviation from the growth rate of the original transplanted tumor, or evidence of further metastatic activity.

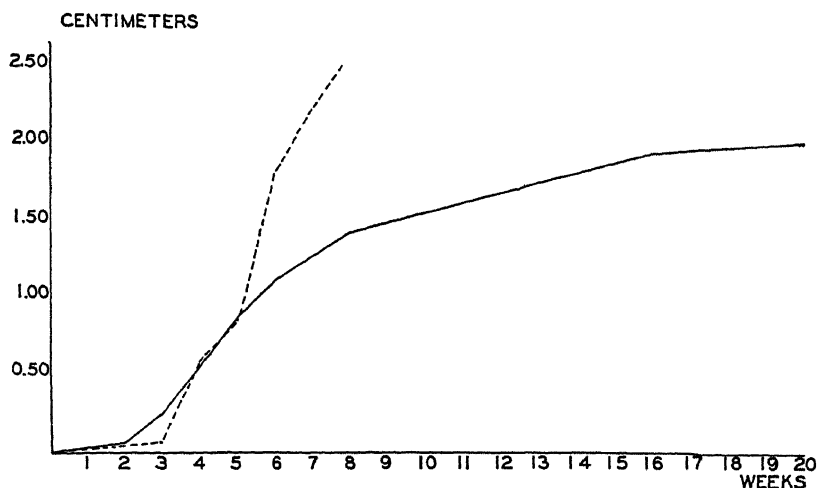


FIGURE 6.—The average growth rate of the transplant in (a) male mice (solid line) and (b) male mice receiving follicle-stimulating hormone (dash line).

The injection of the follicle-stimulating hormone had no detectable effect on the growth rate of the tumor or the survival time of normal female mice. On the other hand, it had a significant stimulating effect on the transplant growth in normal males (fig. 6).

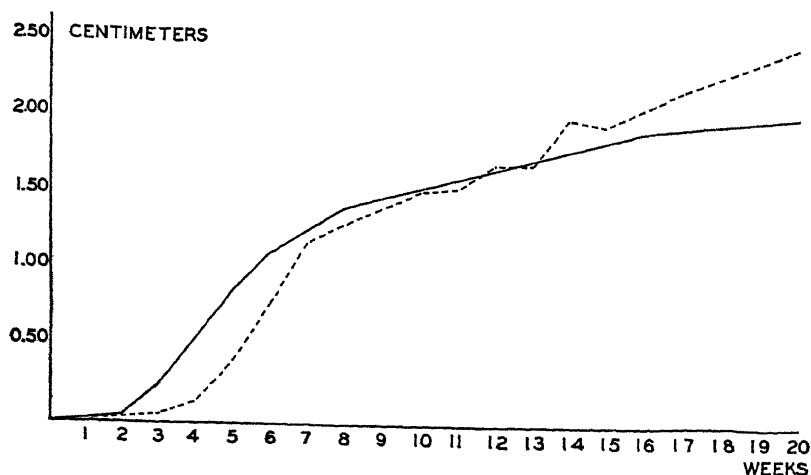


FIGURE 7.—The average growth rate of the tumor in (a) male mice (solid line) and (b) male mice receiving prolutin (dash line).

Prolutin may have had a slight inhibitory action on the growth of the carcinoma of the ovary, since the growth curve lagged behind that ob-

tained for the controls—and eventually approached the normal curve after the injections of prolutin were discontinued (fig. 7).

The injection of estrogen into both males and females inhibited the growth rate of the transplanted tumors (figs. 3 and 8).

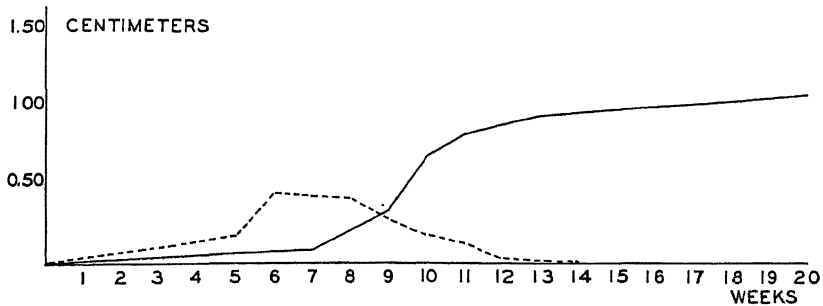


FIGURE 8.—The average growth rate of the transplant in (a) female mice (solid line) and (b) female mice receiving estrogen (dash line). Female mice receiving estrogen completely regressed their tumors.

The growth rate of the implanted tumor was not altered by the injection of any of the hormone preparations when the individual (male or female) had been previously castrated. The injection of egg albumen did not affect the growth rate of the tumor. The comparative effect obtained by the various injections is given in table 4.

TABLE 4  
FEMALE

|              | FEMALE |          | MALE   |          |
|--------------|--------|----------|--------|----------|
|              | NORMAL | CASTRATE | NORMAL | CASTRATE |
| Mare's Serum | +      | o        | o      | o        |
| F.S.H.       | o      | o        | +      | o        |
| B.D.H.       | —      | o        | —      | o        |
| Prolutin     |        |          | —      |          |
| Egg Albumen  | o      | o        | o      | o        |
| Male Hormone |        |          |        | o        |

#### GENERAL DISCUSSION

Though this tumor arose in the ovary a delayed or complete failure of its development following transplantation into intact female mice soon became apparent. Whether the tumor arose under altered hormonal conditions or underwent changes following transplantation afforded two possibilities for speculation.

Since the genetic study indicated a non-genic sex-limited regulation of transplantation and rate of growth the possibility of hormonal factors was investigated. The intact male afforded the optimum environment for the transplant; the intact female the least satisfactory host. Orchidectomy inhibited the growth of the tumor.

The increased rate of growth of the transplanted tumors in normal females receiving pregnant mare's serum might indicate that this effect was obtained by the action of the ovary. F.S.H. was, however, without effect. The failure of estrogen or progestin injections were not compatible with such interpretation. The lack of effect of pregnant mare's serum in intact males indicates an absence of any direct effect of this gonadotropic principle. The present experiments fail to explain definitely the role of endocrines indicated by the differences of growth of the transplant in the males and females. Some tumors particularly certain adenomas of genital tissues respond to their hormonal environment. The more malignant tumors have failed to respond to most hormonal deficiencies, presence or excesses, unless the general condition of the animal is greatly affected. It is particularly interesting that this comparatively malignant tumor has also retained to some extent the physiological activity of the tissue of origin.

The tendency of the tumor to calcify, particularly when growing slowly, in intact females also merits further investigation.

The genetic theory of transplantation of neoplastic tissue as postulated by STRONG formulated the conception that the fate of neoplastic tissue within the body was controlled by a reaction between the tumor and the host. The reactivity of the host is controlled by its intrinsic genetic constitution; that of the tumor is also determined by an internal genetic constitution. The data of STRONG, BITTNER and CLOUDMAN have clearly demonstrated that the genetic constitution of the tumor may change from time to time presumably by a process similar to somatic mutation.

The present data, on the other hand, demonstrate that the reactivity of the host toward a transplantable carcinoma of the ovary may be influenced by the injection of hormone solutions or by gonadectomy.

The genetic data demonstrate that susceptibility and resistance to the transplant are determined intrinsically by multiple mendelian factors. There are at least two hypotheses for the explanation of the relationship between genic and hormone action. The first one maintains that the early development of the individual is determined by genic action. This is eventually replaced by hormone action during the adult physiological life of the individual. The second hypothesis maintains that genic action and hormone action are both present simultaneously. With the accumulated data on the effect of genes throughout the entire life span of the individual and on the inheritance of disturbances of the endocrine system itself, it seems more likely that hormone action may very well be controlled by or be concomitant with genic action.

It is more than probable therefore, that in this investigation the influence of hormone preparations on susceptibility and resistance to a transplantable carcinoma of the ovary has been demonstrated by the possibility

that the defect or variation received by the individual through its germ plasm has been made good by hormone therapy.

#### SUMMARY

The growth of a transplanted carcinoma of the ovary depends on the simultaneous presence of multiple mendelian factors (probably four). The growth rate of the tumor can be influenced, however, by internal secretions of the host. However, the endocrine factors have not been clearly worked out because of (1) the extreme calcification undergone by the tumor and (2) the extremely slow growth of the transplant in intact females.

The problem of tumor susceptibility and resistance is discussed; the interest inheres primarily in the fact that the tumor has retained the physiological activity of the tissue from which it originated (that is, the ovary).

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# SOME EFFECTS OF X-RADIATION ON THE NEUROBLAST CHROMOSOMES OF THE GRASSHOPPER, *CHORTOPHAGA VIRIDIFASCIATA*

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Received July 27, 1938

RECENT studies and interpretations of the genetic effects of X-ray induced translocations and inversions in plants and animals emphasize the need for additional information concerning the cytological aspects of such chromosome alterations. Realization of this has prompted a number of investigators (LEWITSKY and ARARATIAN 1931; MATHER and STONE 1933; STONE 1933; MATHER 1934, 1937; HUSKINS and HUNTER 1935; WHITE 1935, 1937; RILEY 1936; LEVAN 1936; GUSTAFSSON 1937; and NEBEL 1936, 1937) to undertake studies of the immediate effects of X-rays on chromosome morphology. Because the results of these studies, though agreeing in many essentials, nevertheless seem utterly opposed and contradictory in other respects, further work is needed, utilizing other and, as far as possible, more favorable material.

The neuroblasts of the embryo of the grasshopper, *Chortophaga viridifasciata*, were used in the present study. These cells are particularly suitable for observations of chromosome form and alteration (CARLSON 1937). They are present in relatively large numbers. They are readily identifiable because of their large size, which is sufficient to accommodate the metaphase and anaphase chromosomes with a minimum of crowding and overlapping; this is especially important in X-rayed material, in which one must be able to distinguish between translocation and juxtaposition. Also, like the better-known germ cell chromosomes of the grasshopper, these somatic chromosomes are large. In this species all are telomitic, and so have the form of simple rods. On the other hand, a disadvantage of this material, especially as compared with the microspores of certain plants, is the large number of chromosomes (23 and 24 in the male and female, respectively), which makes it impossible to identify and interpret changes in terms of individual chromosomes, after even a relatively slight X-ray treatment.

My thanks are due DR. A. F. BLAKESLEE for the opportunity of working at the Department of Genetics of the Carnegie Institution of Washington during the summers of 1937 and 1938. I am deeply indebted to DR. B. P. KAUFMANN and DR. M. DEMEREC for much valuable discussion as well as many helpful suggestions and criticisms relating to this study.



## MATERIAL AND METHODS

Eggs containing embryos 3-5 weeks old were given X-ray dosages of 100, 125, 250, 500, 750, and 1000 r.<sup>1</sup> At the end of certain time intervals after irradiation embryos were removed from the eggs and made into permanent slides by BAUER'S (1936) modification of the aceto-carmine method. This consists of immersion for 30-40 minutes in aceto-carmine, spreading on an albuminized glass slip under a greased cover glass, removal of the cover glass in 95 percent alcohol, and mounting directly in euparal.

It is true that fixation and staining with aceto-carmine do not give the uniformly and consistently excellent chromosome preparations that many other technics do. The ease and speed that it affords, however, recommend its use where a large number of preparations must be made in a limited period of time, provided, of course, that the shortcomings of the method be kept in mind in interpreting results. The aceto-carmine method described above gives preparations that are adequate for the purposes of the present study.

## EFFECTS ON MITOSIS

In the normally developing embryo at any given time different neuroblasts are in different stages of mitosis. Following irradiation there is a rapid disappearance of middle prophase through telophase stages. At the end of one and a half hours, after 500 r, only interphases and early prophases are left. This cessation of mitosis persists for a period of time varying with the X-ray dosage. Anaphases first begin to reappear after about 3, 7, 17, and 22 hours following dosages of 100, 250, 750, and 1000 r, respectively. Additional data on this effect will be included in a later paper.

The present study is based primarily on material fixed within a few hours of the end of the period of cessation, in order to obtain stages of the same mitotic cycle in which irradiation occurred. Since the mitotic cycle of the neuroblast requires at least two days, and probably often more, for its completion, alterations dealt with in the present paper are solely immediate effects.

## CHROMOSOME ALTERATIONS

The findings of several investigators support the view that X-radiation may alter the chromosomes at any stage of the mitotic cycle. These changes are much more drastic, however, when the chromatin is diffuse, that is, in telophase through early prophase, than in stages in which it is

<sup>1</sup> In view of the recent conclusion of Gustafsson (1936, 1937) that in plant root tips the frequency of chromosome X-ray effects increases with the increase in water content of the tissues, it seems important to remark that all eggs used in the present study were kept saturated with water for at least several days previous to treatment.

concentrated in well-formed late prophase, metaphase, and anaphase chromosomes. MATHER and STONE (1933), WHITE (1935), RILEY (1936), and GUSTAFSSON (1936, 1937) found alterations in cells irradiated at interphase and early prophase stages. In addition RILEY reported breaks produced in late prophase and metaphase chromosomes. HUSKINS and HUNTER (1935) described chromosome and chromatid fragmentation and translocation in cells treated at telophase.

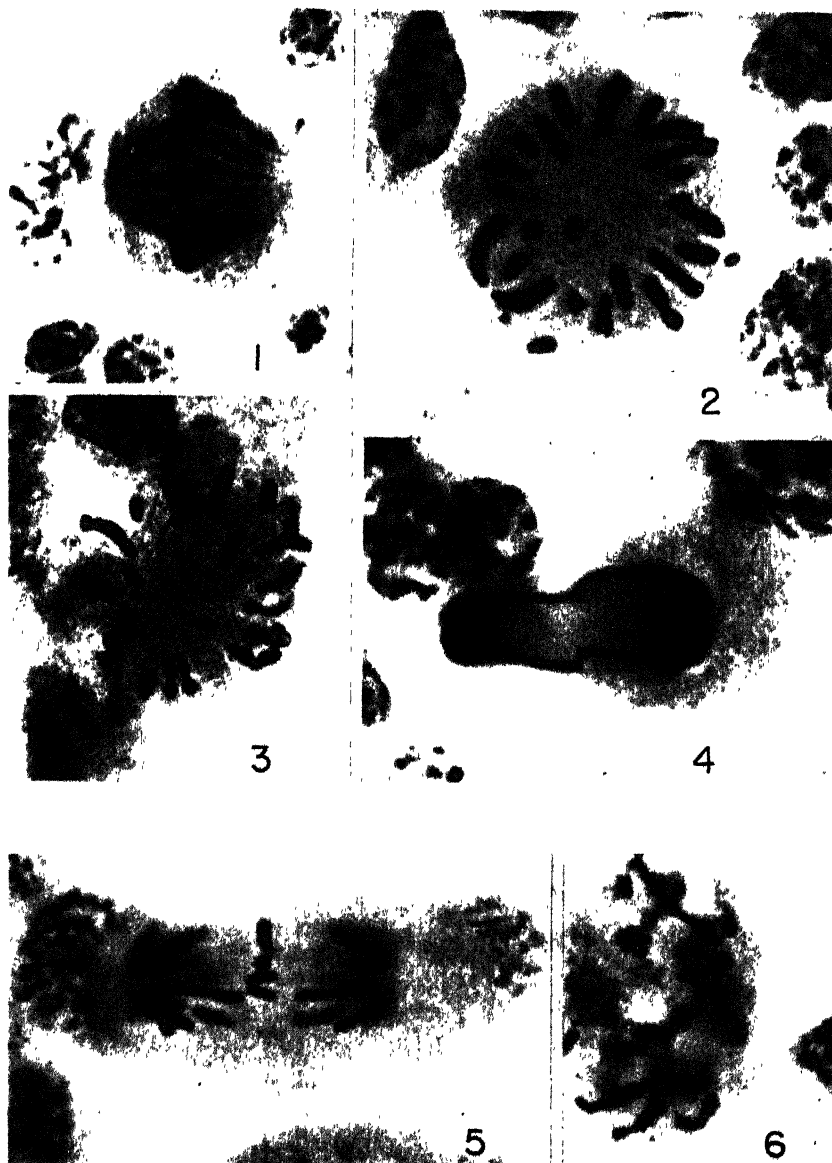
The chromosomes of mitotically active neuroblasts fixed within a few minutes after irradiation differ to some extent from normal, untreated ones. In late prophase sister chromatids appear to be very closely approximated at intervals along their length, these places having the appearance of constrictions. Metaphase chromosomes are longer than normal, exhibit severe constrictions, and show considerable twisting of sister chromatids about each other. Anaphase chromosomes appear joined together distally, while proximally they are elongated and show unusually broad constrictions (fig. 1).

X-ray effects that have occurred between telophase and mid-prophase, as observed in cells fixed in the subsequent metaphase and anaphase stages, show not only chromosome alterations but also chromatid and what may be half-chromatid inequalities. No evidence of either increase or decrease in the number of spindle fiber attachments was found in any of the cells in which accurate counts could be made.

In the following pages the term *fragment* is applied to a portion of a chromosome resulting from one or more breaks in an original chromosome and having no spindle fiber attachment. Either unaltered chromosomes or altered ones that have at least one spindle fiber attachment are referred to as *chromosomes*.

### 1. Chromosome Fragmentation

The fragments resulting from chromosome breakage are observable in the metaphase immediately following X-radiation. They are distinguishable from chromosomes with spindle fiber attachments, which are arranged with their kinetochores in an even circle and their distal ends extending outward, because they typically lie outside these chromosomes and have a less regular orientation (fig. 2). At early anaphase the "chromatids" of fragments, though lacking spindle attachments, separate at the same time as those of the chromosomes, which possess kinetochores. This separation may be complete or incomplete, but its occurrence proves conclusively that the initial anaphase separation of chromatids is entirely independent of the kinetochore (CARLSON 1938) and is probably autonomous. A more complete account of the behavior of fragments during mitosis is now in course of preparation.



FIGURES 1-6 --Photographs of irradiated neuroblast chromosomes. Length of time after irradiation: figure 1--about 3 min.; figure 5--12 hrs.; figures 3, 6--23 hrs.; figure 2--25 hrs.; figure 4--166 hrs. Dosage: figures 2, 4, 5--250 r; figure 1--500 r; figures 3, 6--750 r.  $\times 1100$ .

FIGURE 1.—Anaphase. Pronounced constrictions present immediately after irradiation.

FIGURES 2, 3.—Metaphases. Fragments at periphery of cells. Note translocations in figure 3, one of which is shown in figure 16.

FIGURE 4.—Anaphase. Very long translocated chromosomes.

FIGURE 5.—Anaphase. Two V-shaped fragments in equatorial plane.

FIGURE 6 —Late prophase. Chromatid translocation. For details see figure 8.



The number of fragments per cell varies with the X-ray dosage. After 125 r there are rarely more than three or four fragments per cell, and frequently none at all. On the other hand, after 1000 r there may be more than thirty fragments per cell and no cells lacking them entirely. Because fusion of fragments *inter se* and with chromosomes obviously occurs with much frequency, counts of independent fragments give no accurate information of the actual amount of breakage originally effected by treatment, and so were not attempted.

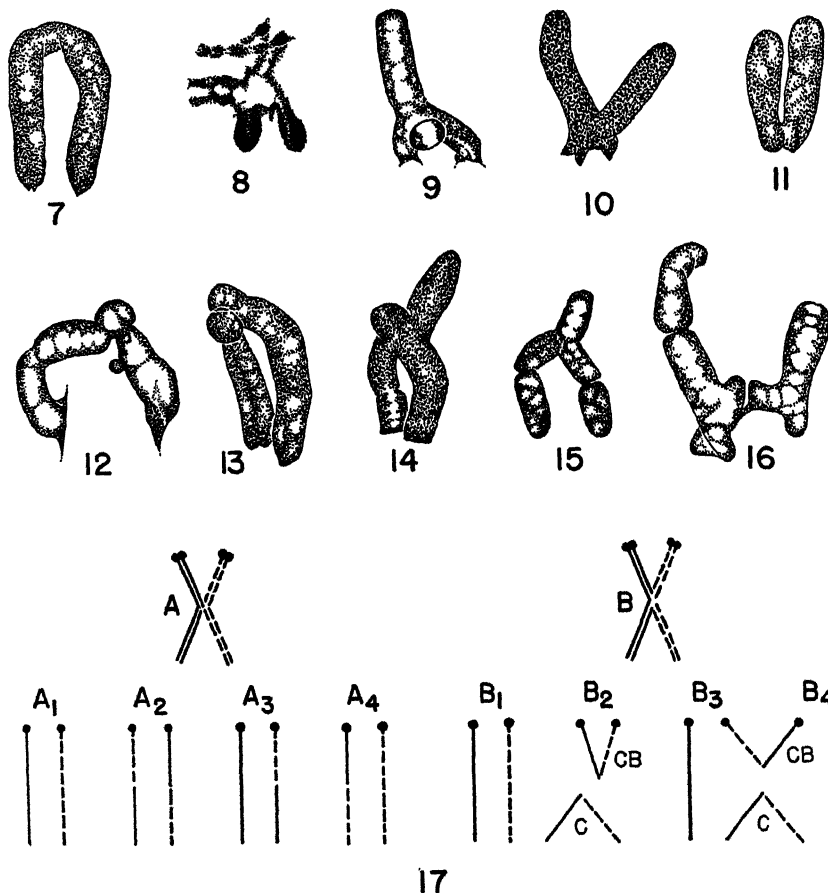
## 2. Chromosome Translocation

The large number of chromosomes per cell and lack of morphologically distinguishing characters make impossible any attempt to determine the number and extent of the chromosome translocations involved after irradiation.

Positive proof of translocation exists in the form of abnormally long chromosomes (fig. 4) and U-shaped chromosomes with two or more kinetochores (fig. 7). The anaphase behavior of the latter depends on the orientation of the kinetochores on the spindle (MATHER and STONE 1933; HUSTED 1936). If both spindle fiber loci of fused chromatids are directed toward the same pole, and the chromatids are not twisted about each other, a U-shaped daughter chromosome will move toward each pole (figs. 22, 23). If there is a half-turn in the chromosome, however, so that the spindle fiber loci of each fused chromatid point toward opposite poles, the anaphase configuration will have the form of two crossed chromatin bridges (figs. 24, 25). U-shaped chromosomes may persist as such, therefore, from one cell generation to the next, until they become oriented on the spindle with a half-turn from end to end. A delayed effect, resulting from the breakage of the crossed chromatin bridges and the subsequent formation of new attachments at the broken ends, might appear, as a consequence, a considerable number of cell generations after irradiation. The ultimate fate of chromatin bridges is considered in detail in a later section.

## 3. Chromatid Inequalities

Neuroblast chromosomes fixed and stained by most methods have, at metaphase and anaphase, a homogeneous appearance that affords no evidence of internal structure. In aceto-carminic smears, on the other hand, chromosomes in these stages usually exhibit a lightly staining "matrix" containing darker-staining regions comparable to the chromatids of the prophase (compare figs. 8 and 9). The metaphase chromosome and fragment each contains two such parallel regions (figs. 2, 3, 9, 12, 16, 26, 27). The anaphase chromosome and fragment each shows one—it may be double—lying in the middle of the chromosome and extending from end



FIGURES 7-17.—Irradiated chromosomes with more than one spindle attachment as a result of translocations. Spindle attachment ends, except in figure 17, are directed toward bottom of page. Figure 8 is in late prophase; others in metaphase. Length of time after irradiation: figures 13, 14—10 hrs.; figures 7, 15—12 hrs.; figures 11, 12—18 hrs.; figures 8, 9, 10, 16—23 hrs. Dosage: figures 1, 7, 8, 9—250 r; figure 5—500 r; figures 2, 3, 4, 6, 10—750 r.  $\times 2475$ .

FIGURE 7.—Chromosome translocation.

FIGURES 8-16.—Chromatid translocations. Figures 8 and 16 appear as photographs in figures 6 and 3, respectively.

FIGURE 17.—Diagram of chromosomes with different types of chromatid translocations, showing the possible distribution of their chromatids at anaphase. A (diagram of figure 9) will give combinations A<sub>1</sub> and A<sub>2</sub> or A<sub>3</sub> and A<sub>4</sub> at anaphase. B (diagram of figures 6 and 8) will give combinations B<sub>1</sub> and B<sub>2</sub> or B<sub>3</sub> and B<sub>4</sub>. CB, chromatid bridge. C, "chromatid" lacking spindle attachment. Knobs at ends of chromosomes and chromatids indicate positions of spindle attachments.

to end (figs. 5, 18, 28-31, 33-37). Although the appearance of these regions suggests that they are tightly coiled chromonemata, it seems best, until they have been examined after other treatment, to refer to them and the "matrix" around them non-committally as chromatids.

HUSKINS and HUNTER (1935) have demonstrated that some of the chromosomal constrictions so numerous in irradiated material are actually chromatid breaks. Certain X-ray induced constrictions present in my material appear to be the result of incomplete fusions of chromosomes, the ends of one chromatid of each chromosome having united and the other chromatids having remained unattached. A fragment of this type appears in figure 37. In the ensuing anaphase the distal portion of the interrupted chromatids will constitute a chromatid fragment (fig. 18 A). The non-corresponding constrictions exhibited by the daughter chromosome pairs shown in figures 18 A, 19, and 21 appear to be chromatid inequalities, but may possibly represent half-chromatid effects.

A frequently observed type of chromatid inequality in my material is what may be called a chromatid translocation (figs. 3, 8-17), namely, the "lateral translocation" of HUSKINS and HUNTER (1935), "reciprocal chromatid fusion" of WHITE (1935), or "pseudobivalent" of LEVAN (1937). The fusion may give either of two configurations. (1) If the proximal portion of one chromatid of each of the two chromosomes involved is joined to the distal portion of the other chromosome, each of the four chromatids will have a single kinetochore (figs. 9, 17 A), and the configuration will resemble the cross-shaped diakinetid tetrad. At anaphase two chromatids will pass to each pole, the orientation of the kinetochores on the spindle determining whether both (fig. 17, A<sub>1</sub> and A<sub>2</sub>) or neither (A<sub>3</sub> and A<sub>4</sub>) of the daughter cells will get a complete complement of chromosomes. (2) If the two proximal and the two distal portions are joined *inter se* at the point of breakage, the chromosome will consist of two unaltered chromatids, one chromatid with two kinetochores, and one with none (figs. 6, 8, 17 B). If the unaltered chromatids pass to the same pole (fig. 17 B<sub>1</sub>), the one with the two kinetochores will pass to the other pole (B<sub>2</sub>); but if the former pass to opposite poles (B<sub>3</sub> and B<sub>4</sub>), the kinetochores of the latter will also move toward opposite poles, thus forming a chromatin bridge (CB). In each case the "chromatid" without a kinetochore (C) may pass into either of the daughter cells. Only one of these four possible daughter cells will possess a complete set of chromosomes, namely, the one into which the two unaltered chromatids pass.

#### 4. *Half-chromatid Inequalities*

NEBEL and RUTTLE have concluded from observations of the somatic chromosomes of *Tradescantia* (1935, 1936) and *Hordeum*, *Secale*, and *Crocus* (1937) that doubling of the chromonemata occurs two generations in advance of their anaphase separation, so that from one anaphase through the next prophase each chromatid is composed of two chromonemata or half-chromatids. I have found a few figures in my prepara-

tions that seem very suggestive of half-chromatid effects, though none furnishes what I consider to be entirely convincing proof of the occurrence of such inequalities. This is due in part to the nature of this problem, itself, and in part to the fact that I have looked for these only in stages of the division immediately following irradiation, while the presence or absence of half-chromatid effects should be more readily detectable in the second division following irradiation.

In figures 18 A, 19, and 21 are shown anaphase pairs of daughter chromosomes that were irradiated in the interphase or early prophase condition. They exhibit constrictions at non-corresponding places. That such constrictions are not comparable to the secondary constrictions normally present in untreated material of many different organisms is evident from the fact that, in the latter, sister chromatids are constricted at corresponding points. They may, however, be comparable to the constrictions in metaphase chromosomes resulting from a break in one of the two chromatids (HUSKINS and HUNTER 1935), but, because they occur in anaphase instead of metaphase chromosomes, they would represent, by analogy, half-chromatid breaks instead of chromatid breaks.

One member of each of pairs A and B (fig. 18) displays a short region of abnormally small diameter. Comparison of the lengths of each of these with its sister chromosome suggests that the segment of small diameter has been inserted, its small size being due, perhaps, to the possibility that it is an inserted half-chromatid.

The lower chromosome of pair A (fig. 18) and one "chromatid" of the fragment shown in figure 34 have small blobs of chromatin protruding from the side. Underneath each and corresponding to it closely in size is a lightly staining area. It seems not unlikely that each little blob may be a piece of a half-chromatid that has been "knocked out" of the central part of the chromatid, but has remained connected with it peripherally.

#### CHROMATIN BRIDGES AND FRAGMENTS

The fact that X-ray studies of *Drosophila* salivary gland chromosomes have as yet failed to disclose a single positive case of a terminal inversion has led many to hold that a broken end of a chromosome cannot exist as such, at least over the course of many cell generations, and that, therefore, if a single break occurs, it is somehow eliminated. It is generally assumed that some kind of unsatisfied attraction causes broken ends of chromosomes to unite with one another. In the cell shown in figure 2 an original set of 23 chromosomes with 46 ends has been altered by irradiation to give a total of 26 chromosomes and fragments possessing together 52 ends. This and many other similar examples leave no doubt that new ends, whether of chromosomes or fragments or both, may be formed as a result of X-radiation.



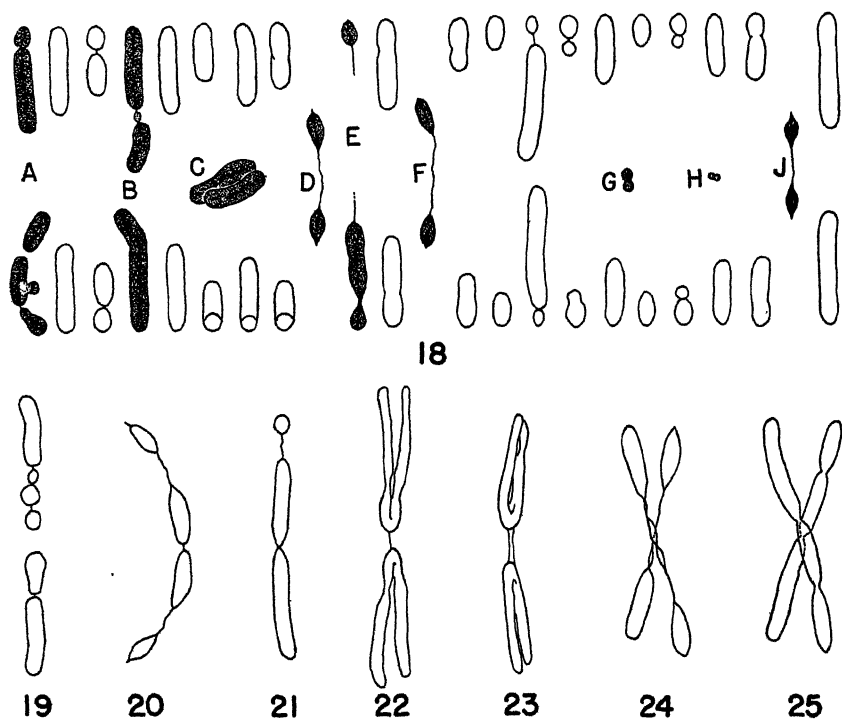
My material suggests, however, that the presence of such new ends does not actually demonstrate the existence of new, unsaturated ends of gene strings. It appears, instead, that in these instances the broken ends of sister chromatids of chromosomes and fragments have fused *inter se*. This assumption makes understandable two classes of chromosomal forms observed at anaphase, namely, chromatin bridges and fragments of three kinds.

A lagging at anaphase of certain daughter chromosomes and great attenuation of their distal ends to form chromatin bridges (fig. 18, D, F, J) result from failure of these ends to separate normally. It seems probable that the distal ends of the chromatids of such a chromosome are newly formed ends, which, unable to satisfy their attraction for broken ends of the chromatids of another chromosome, have fused with one another and tend to retain this union even at anaphase (fig. 38 CB).

The chromatin bridges shown in figure 18 (D, F, J) and in figure 20 may be expected to part at the site of the former break, so that equal daughter chromosomes with the same broken ends will pass into different daughter cells. If in the next cell generation the broken ends of the sister chromatids are again fused, chromatin bridges will be formed anew. Such broken ends can certainly, therefore, be transmitted from one cell generation to the next and may be supposed to persist in this manner from cell generation to cell generation until one of the following events occurs. (1) The broken end may unite with the broken end of a fragment at some subsequent cell generation to produce a normally behaving translocated chromosome (the "delayed attachment" of STADLER 1932). This presupposes that fragments may pass from parent to daughter cells, and this frequently occurs in these cells (CARLSON 1938). (2) The broken end may unite with the broken end of another chromosome to form a U-shaped chromosome with two spindle attachments. The mitotic behavior and probable ultimate fate of these has been described above. (3) The broken end may eventually acquire the properties of a true end, and so the chromatids cease to fuse at their broken ends to form chromatin bridges. Such chromosomes will then become normally behaving chromosomes with terminal deficiencies. This possibility is suggested by the X-chromosome deficiencies described by DEMEREC and HOOVER (1936) in *Drosophila*. (4) Sufficient genic material may be lost in the remnants of the chromatin bridge left outside the nucleus at the telophase of several succeeding divisions to produce a deficiency that is lethal to the cells. (5) The point of breakage of the chromatin bridge does not always occur at the same place, that is, at the point of chromatid fusion (fig. 18 E, 21), so that a deficiency is produced in one cell and a duplication in the other. Either or both may be cell lethal. The importance of any of these changes from the

genetic aspect will, of course, depend on whether or not the cell survives them; for, if it does not, the altered chromosomes will be lost with the cell.

Chromatin bridges have been observed in a number of different organisms in the first meiotic anaphase. They have been interpreted as chromatids with two spindle fiber loci resulting from crossing over in the inverted



FIGURES 18-25.—Anaphase separation of daughter chromosomes following irradiation. Length of time after irradiation: figures 19, 21—12 hrs.; figure 20—22 hrs.; figure 18—23 hrs.; figure 24—47 hrs.; figure 22—75 hrs.; figure 25—119 hrs.; figure 23—143 hrs. Dosage: figures 18, 19, 21—25—250 r; figure 20—500 r.  $\times 1585$ .

FIGURE 18.—Complete set of anaphase chromosomes and fragments. A and B, chromatid or half-chromatid (?) inequalities. C, G, H, fragments. D, E, F, J, chromatin bridges.

FIGURE 19.—Chromatid or half-chromatid (?) inequality.

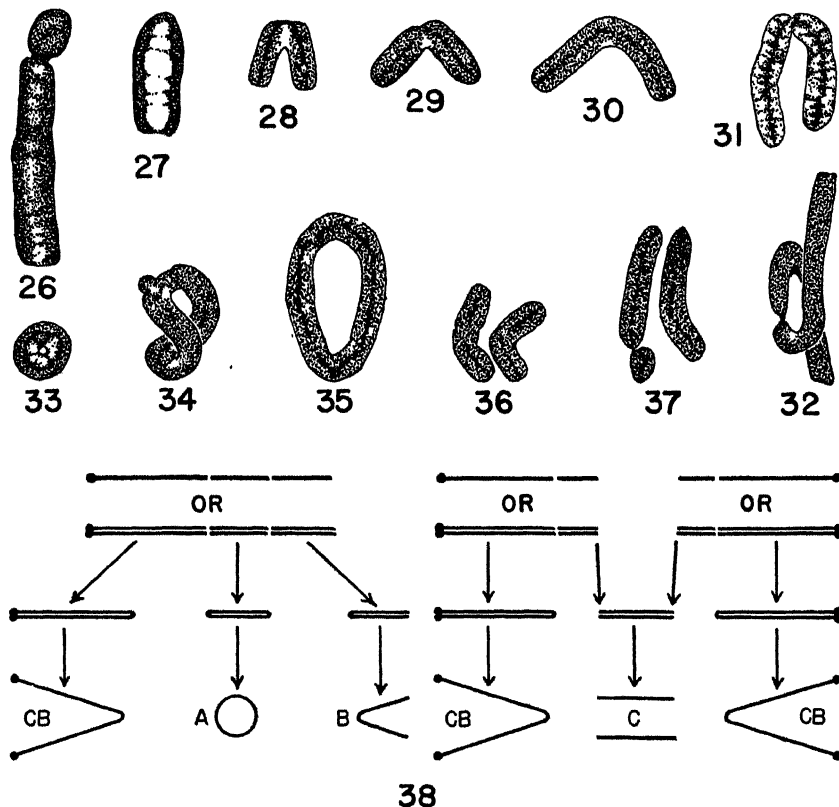
FIGURE 20.—Chromatin bridge.

FIGURE 21.—Chromatid or half-chromatid (?) inequality and chromatin bridge.

FIGURES 22-25.—Types of separation of chromosomes with two spindle attachments.

portion of chromosomes in inversion heterozygotes. In the microspores studied by HUSTED (1936) and in my material, however, another explanation has been necessary to account for these chromatin bridges, since synapsis and crossing over cannot have been involved. To sum up, the conclusion seems justifiable that the chromatin bridges in my material have arisen in three different ways: (1) from chromosome translocation,

(2) from chromatid translocation, and (3) from fusion of sister chromatids at their broken ends.



FIGURES 26-38.—Autonomous separation of "chromatids" of X-ray produced fragments, which lack spindle attachments. Length of time after irradiation: figures 27-32, 35, 36—12 hrs.; figure 26—13 hrs.; figures 33, 37—21 hrs.; figure 34—23 hrs. Dosage: figures 26-32, 35, 36—250 r; figures 33, 34, 37—750 r.  $\times 2475$ .

FIGURE 26.—Metaphase fragment.

FIGURE 27.—Early anaphase. Beginning of separation of "chromatids" except at one end.

FIGURES 28-32.—Middle anaphase. V-shaped fragments resulting from "chromatid" separation except at one end. See figure 38 B.

FIGURES 33-35.—Anaphase. Ring-shaped fragments resulting from separation of "chromatids" except at both ends. See figure 38 A.

FIGURES 36, 37.—Anaphase. Complete separation of "chromatids" to give two rod-shaped fragments. See figure 38 C.

FIGURE 38.—Diagram showing types of breakage and fusion that lead to formation at anaphase of chromatin bridges (CB) and fragments having the form of V's (B), rings (A), and double rods (C). Knobs at ends of chromosomes and chromatids indicate location of spindle attachments.

During early anaphase the "chromatids" of fragments may behave in any of three different ways<sup>2</sup> (CARLSON 1938). First, they may separate

<sup>2</sup> This classification does not include the small spherical fragments that are present in most cells after treatment.

except at one end to form a V (figs. 5, 27-32). Second, they may remain connected at both ends and separate centrally to form a ring (figs. 33-35). Third, they may separate completely to form two rods lying side by side (figs. 36, 37). The fact that the "chromatids" of fragments, which lack kinetochores, begin to separate with the advent of anaphase, at the same time as the chromosomes with kinetochores, indicates that separation is an intrinsic character of the chromosome. Their tendency to remain attached frequently at either one or both ends, however, points not only to some fundamental difference in the ends as compared with the central parts of the fragments, but also to differences in the ends of the fragments. A given fragment end may be either the original end of the chromosome of which it was once a part, or a new one created in the breakage of the original chromosome at that point. The "chromatids" of a terminal fragment would be expected to fuse with one another at their broken ends. At anaphase these ends would remain fused, as the opposite true ends separated, to give a V-shaped fragment (fig. 38 B). Fusion would be expected to occur at both ends of an intercalary fragment, and the retention of this union after the central portion of the "chromatids" had separated would give the ring-shaped anaphase fragment (fig. 38 A). Finally, if a given fragment were the result of fusion at the broken ends of the terminal fragments of two original chromosomes, the remaining ends would be true ends, and so the "chromatids" would be expected to separate completely at anaphase to form two distinct rods (fig. 38 C). V's are the most abundant type. Next in frequency are the two rods. Rings occur only rarely at dosages below 500 r, but frequently at 750 and 1000 r.

In figure 18 three fragments (C, G, H) and four chromatin bridges (D, E, F, J) appear. According to the above hypothesis they would be explained thus. Fragments G and H are assumed to represent the true ends of two of the chromosomes connected by bridges. Fragment C is separating as two rod-shaped chromatid fragments. Both ends are, therefore, true ends, having resulted from the fusion, at their broken ends, of two distal fragments from the other two chromosomes with bridges.

UPCOTT (1937) and BARBER (1938) have reported chromatin bridges in microspores lacking fragments. The latter has shown, however, that aging of the pollen somehow causes a fusion of true ends of sister chromatids.

#### DISCUSSION

*Time of chromosome doubling.* The conclusions of different cytologists regarding the time of doubling, or splitting, of the chromosomes have recently been summarized by GUSTAFSSON (1936) and KAUFMANN (1936). Views range all the way from doubling in the late interphase or early pro-

phase immediately preceding anaphase to doubling in the prophase one mitotic cycle in advance of anaphase separation. Most of the chromosomes on which my results are based were probably in late interphase or early prophase at the time of X-radiation, and both chromosome and chromatid effects were produced. If the half-chromatid effects suggested by my material are valid, doubling has occurred in the late interphase or early prophase one mitotic cycle in advance of anaphase separation of the units thus formed. If one disregards these, however, there remains no important evidence bearing on this problem.

*Mechanism of chromosomal change.* According to the hypothesis proposed by SEREBROVSKY (1929) X-rays effect structural changes in the chromosomes by causing fusions of different chromosomes or different parts of a single chromosome, which is followed by breakage in a different plane. The proposal of STADLER (1932) is just the reverse of this; fusion is supposed to follow breakage rather than precede it. If it may be assumed that in either hypothesis the first occurrence conditions the second, an increase in the total number of chromosomal elements, namely, chromosomes and fragments, which is invariably the situation in cells showing any effects at all, seems to support the concept of breakage preceding fusion. In none of the affected cells in my material is there any decrease in the number of chromosomal elements, which would result if there were a predominance of fusion over breakage. Another difficulty of the SEREBROVSKY hypothesis is its dependence on close proximity or contacts between chromosomes at the time of effective irradiation, that is, between late telophase and early prophase. This requirement may be realized in many types of cells. The grasshopper neuroblast, however, is an unusually large cell, containing a large lobed nucleus with a central cytoplasmic core. The ends of three or four of the longer chromosomes extend distally into each of five or six lobes. Contacts between different chromosomes, therefore, are considerably limited. It seems improbable that a sufficient number of contacts could exist between these chromosomes at the time of irradiation to account for the complex fusion configurations present in many of these cells after treatment with 1000 r, unless it is assumed that treatment, itself, causes extreme movement of the chromosomes with the establishment of new contacts as treatment progresses. Of particular interest in this connection is a questionable translocation involving the X-chromosome and an autosome in figure 15 of WHITE's 1935 paper on *Locusta*. The X chromosomes of many Acrididae, and, therefore, probably of *Locusta*, lie in vesicles apart from the other chromosomes from telophase through late prophase. The irradiation bringing about this translocation doubtless occurred during this period (this cell was fixed 17 hours after treatment),

and breakage could have occurred at that time. Fusion with the autosomal piece must have occurred subsequently, however, after the disappearance of the membrane of the vesicle at late prophase.

#### SUMMARY

The neuroblasts of grasshopper embryos treated with 100, 125, 250, 500, 750, and 1000 r suffer a cessation of mitosis for a period of time proportional to the dosage.

Cells irradiated between telophase and early prophase show, in succeeding stages of the same mitotic cycle, chromosome fragmentation and translocation, chromatid breakage and translocation, and what may be half-chromatid effects.

Chromatin bridges at anaphase appear to result from any of three different alterations: (1) chromosome translocation, (2) chromatid translocation, (3) fusion of sister chromatids of the proximal portions of fragmented chromosomes at their broken ends.

Chromatin bridge formation and the persistence of broken ends of chromosomes from one cell generation to another makes possible delayed reattachments following irradiation.

The early anaphase separation of chromatids, judged on the basis of the behavior of fragments lacking spindle attachments, is entirely independent of the kinetochore, and so is probably an autonomous function of the chromosome.

An hypothesis is suggested to account for three different forms, namely, V's, rings, and rods, which "chromatids" of these fragments assume as they begin to separate at early anaphase.

Certain evidence suggests that chromosomal change is effected by fusion following, rather than preceding, breakage.

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# X-RAY INDUCED CHROMOSOMAL ALTERATIONS IN *DROSOPHILA MELANOGASTER*

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Received July 28, 1938

IN THE fall of 1936 the first two authors began a series of experiments to determine the frequency of minute inversions in material treated by X-rays. Before long it was realized that the frequency of such inversions is very low and that a reliable analysis would involve such technical difficulties that it would require a great deal more time than was available to the authors. Therefore, the original plan was revised and a new experiment started to determine the relation between X-ray dosage and the frequency of chromosomal breaks, the distribution of breaks along the chromosomes, and related problems. Subsequently when the experimental part of the problem was well under way the third author joined in the work.

The first author is responsible for the cytological analysis of the major portion of the female material, the second author was in charge of breeding and X-raying work and helped with the cytological analysis, and the third author is responsible for the cytological analysis of all male material and of a portion of the female data where Swedish-b females were mated with treated Oregon-R males. Permanent preparations of 1765 pairs of salivary glands were used for this study. Slides were prepared by Mr. HERSCHEL ROMAN, Miss RUTH BATE, and Miss EUNICE WHITE, to whom the authors wish to express their appreciation.

When this manuscript was ready for press a paper by CATCHESIDE (1938) appeared describing some of his results obtained in experiments identical with ours. Many conclusions reached by CATCHESIDE are identical with our conclusions.

## MATERIAL AND METHODS

The bulk of the data reported here was obtained from experiments in which inbred Oregon-R wild type stock was used. However, before this experiment was completed a female sterility factor appeared in the stock and to avoid difficulties connected with sterility, females of an inbred Swedish-b wild type line were used in later experiments. In the chart (fig. 1) showing the relationship between X-ray dosage and effects, the data from Oregon-R females are presented separately because differences may exist in the genetic constitution of the females used. In analyses of other problems such differences would probably be unimportant and,



therefore, data from the two sets of experiments were considered together. The X-ray treatment was given to males and they were of the Oregon-R line in all experiments.

The X-ray radiation was applied by an Universal Type Coolidge tube, with a tungsten target, at 85 kilovolts and 7 milliamperes. The X-ray dosage was measured by a Fricke-Glasser dosimeter, manufactured by the Victoreen Instrument Company. In this work a 1/20 cubic centimeter graphite chamber was used by which the total dose applied was measured.

The study was made on salivary gland chromosomes of  $F_1$  offspring from matings between untreated females and treated males. In all cases permanent preparations were made following in general the alcohol-euparal method outlined by BAUER (1936). One individual was available in each instance and in a great majority of the cases that material was sufficient to complete the analysis.

All chromosomal rearrangements were analyzed and breakage points determined within the limits of at least one division of BRIDGES' (1935) map. Small deficiencies involving one division or less were disregarded since they are easily missed in the material available in these studies. There are, however, two types of rearrangements not readily detectable by the method used, namely inversions and reciprocal translocations with both breaks within heterochromatic regions. Frequently unpaired basal sections may be found in non-corresponding chromosome limbs (for example, 2L and 3L) and this may be taken as evidence of such reciprocal translocation. As, however, the closeness of pairing may differ in different individuals, the possibility of detecting such cases is not always granted and their partial inclusion may lead to errors. As there is no reason to suppose that rearrangements of this type behave differently than other rearrangements, they have been omitted completely.

It has not been possible to determine the exact break number in all cases. In one type of altered sperm the aberration consists in the transposition of the nucleolus to a new place somewhere on the chromosome limbs. This probably represents an insertion of the nucleolus-bearing heterochromatic segment of the X or Y chromosome in another position, demanding three breaks (KAUFMANN 1938). Another type of configuration shows a terminal segment of one of the chromosomes attached to the chromocenter. In sperms having the Y chromosome such configurations have usually been analyzed as translocations involving that chromosome. In sperms having the X chromosome the rearrangement possibly represents a translocation involving those proximal ends of the X or fourth chromosomes which correspond to a second "limb" separated from the main body by the spindle fiber attachment as described by KAUFMANN (1934). On the other hand, it cannot always be excluded with certainty

that such cases represent translocations to chromocentral regions of the larger autosomes. These types of changed sperms have been counted as 2-break cases. In other larvae the complete determination of all breaks has not been possible, due to too few clear nuclei, and partly to the combination of changes of the unanalyzable type with other rearrangements. These have been classified for the lowest certain break number. In table 1 they have been registered in brackets behind the total numbers of cases in the respective classes.

## ANALYSIS OF RESULTS

*Number of breaks.*—The number of breaks per changed sperm varies from 2 to 13. No case has been found with one break only which would

TABLE 1  
*Number of sperms with chromosomal aberrations.*

| NUMBER OF BREAKS                      | 1000 r      | 2000 r      | 3000 r      | 4000 r      | 5000 r      |
|---------------------------------------|-------------|-------------|-------------|-------------|-------------|
| 2                                     | 8           | 16 (1)      | 128         | 38          | 47 (3)      |
| 3                                     | 3           | 4           | 22 (1)      | 8 (1)       | 9           |
| 4                                     | —           | 3           | 25 (2)      | 12          | 14 (1)      |
| 5                                     | —           | 1           | 8 (1)       | 1           | 9 (1)       |
| 6                                     | —           | —           | 5           | 2 (1)       | 2           |
| 7                                     | —           | —           | 1           | —           | 4 (1)       |
| 8                                     | —           | —           | —           | 1 (1)       | 1           |
| 9                                     | —           | —           | 1 (1)       | 1 (1)       | —           |
| 10                                    | —           | —           | 1           | —           | —           |
| Undeterminable                        | 1           | —           | 2           | 1           | 1           |
| Total                                 | 12          | 24          | 193         | 64          | 87          |
| Normal sperms                         | 319         | 253         | 532         | 151         | 130         |
| Grand total                           | 331         | 277         | 725         | 215         | 217         |
| % sperms with chromosomal aberrations | 3.63        | 8.66        | 26.62       | 29.8        | 40.09       |
| S. E.                                 | $\pm 1.03$  | $\pm 1.69$  | $\pm 1.64$  | $\pm 3.12$  | $\pm 3.33$  |
| Breaks per changed sperm              | 2.250       | 2.542       | 2.704       | 2.875       | 3.126       |
| S. E.                                 | $\pm 0.125$ | $\pm 0.177$ | $\pm 0.092$ | $\pm 0.180$ | $\pm 0.164$ |
| % breaks per total of sperms          | 8.157       | 22.022      | 72.000      | 85.581      | 125.346     |
| S. E.                                 | $\pm 0.024$ | $\pm 0.045$ | $\pm 0.051$ | $\pm 0.105$ | $\pm 0.123$ |

produce a terminal inversion or a terminal translocation to an unbroken end. Short terminal deficiencies, which might have been viable, have not been observed either. A special experiment was performed crossing Oregon-R males treated with 3000 r-units to attached-X females and studying triple-X female larvae. In addition to the treated X chromosome

such larvae had two untreated ones so that any terminal deficiency induced by treatment would be able to survive. Among 124 larvae examined, none carried a terminal deficiency.

*Relation between dosage and frequency of breaks.*—The relation between the dosage and the percentage of sperm with chromosomal aberrations was determined for 1000 to 5000 r-units at 1000 intervals. A summary of

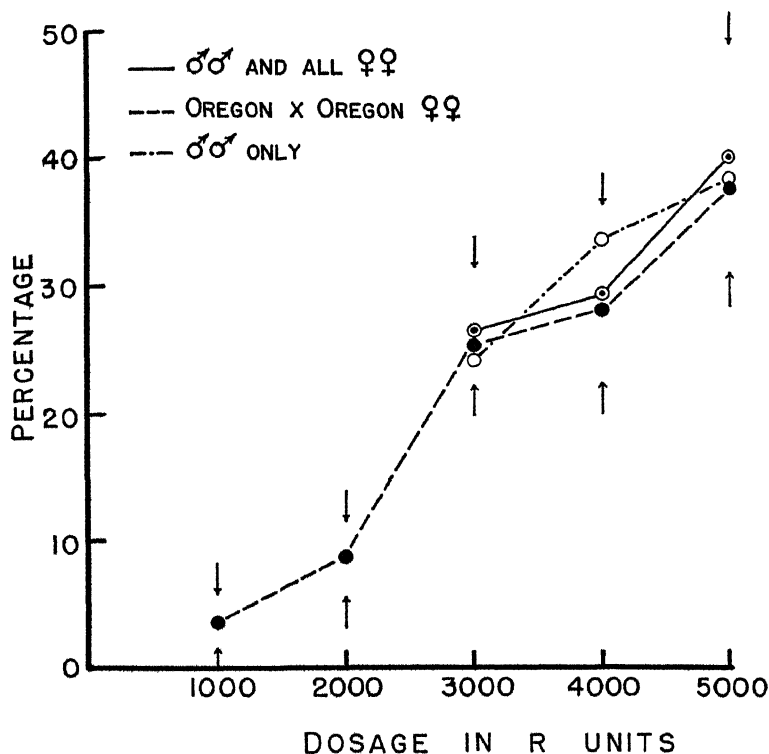


FIGURE 1. —Relation between dosage and the percentage of altered sperm. Arrow tips indicate double values of standard errors.

these data is given in table 1. In this summary is included material from all experiments, namely from females and males with both parents Oregon-R, as well as from females and males of which the female parent was Swedish-b and the male parent Oregon-R. These three sets of data are separated in the graph shown in figure 1. In this figure the relation between the dosage and the percentage of altered sperm is shown graphically. The arrow points on this chart indicate the values of double the standard errors. The curve resembles an S-shaped curve, being steepest in the interval between 2000 and 3000 r-units. Although the standard errors are still high, the observed values show a significant deviation from a linear propor-

tionality obtained in experiments where the frequency of lethals was used as a criterion. Since this is the first occasion in an extensive X-ray experiment that a deviation from linear proportionality is observed, it might be well to wait for confirmation of these results before passing a final judgment as to the significance of this finding.<sup>1</sup> It is evident from the chart shown in figure 1 that the 3000 r-units point is the only one significantly upsetting the straight line arrangement. It might well be that some uncontrolled experimental factor is responsible for the unusual position of that point.

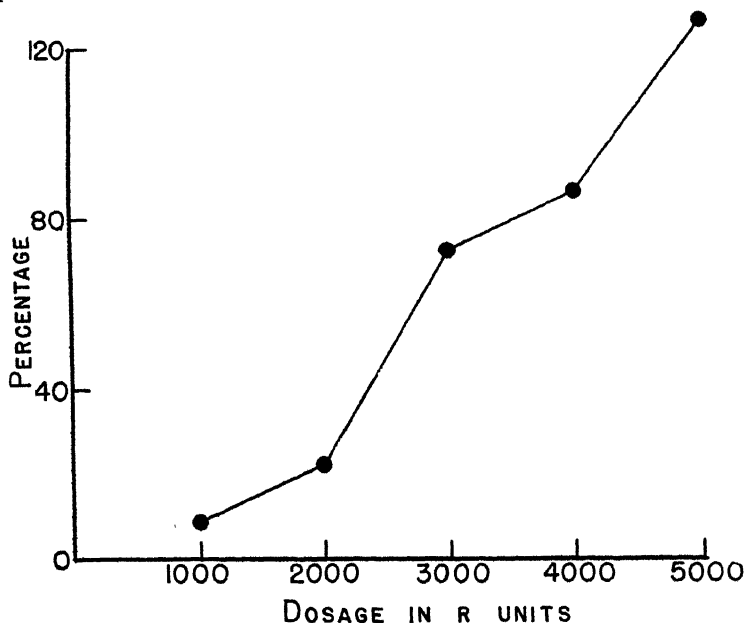


FIGURE 2.—Relation between dosage and the percentage of breaks per total sperms.

The relation between the dosage and the total number of breaks observed is shown graphically in figure 2. The curve rises steeply between 2000 and 3000 r-units and there seems to be no decrease at the highest dosages, although larger numbers would be required to prove this point. These data also show no linear relationship between the dosage and the effect.

The data summarized in table 1 show that with the increase in dosage the proportion of changed sperm increases rapidly. These data indicate also a similar relationship for the average number of breaks per changed sperm. For example, among the changed sperm treated with 1000 r-units,  $2.25 \pm 0.13$  breaks per sperm were observed while among such sperm

<sup>1</sup> Note added in proof: In a paper just published, Sax (1938) has found a similar disproportionality between dosage and observed breaks in irradiated chromosomes of *Tradescantia*.

treated with 5000 r-units,  $3.13 \pm 0.16$  breaks per sperm were found. The data show a trend of increase in the frequency of multiple breaks with increase in dosage, although their absence at low dosages may possibly be due to the low number of total breaks observed in these groups.

*Distribution of breaks among chromosomes.*—The frequency with which the single chromosomes and single chromosome limbs take part in the breakage processes is summarized in table 2. The frequency of X and Y

TABLE 2  
*Break percentage and chromosome length.*

|    | BREAKS IN |          |          |          | TOTAL<br>BREAKS IN<br>AUTOSOMES |      | BREAKS<br>IN X<br>SPERMS | SALIVARY GLAND            |        | MITOTIC                     |                                       | META-<br>PHASE |
|----|-----------|----------|----------|----------|---------------------------------|------|--------------------------|---------------------------|--------|-----------------------------|---------------------------------------|----------------|
|    | X SPERMS  |          | Y SPERMS |          |                                 |      |                          | NO.<br>BANDS<br>(BRIDGES) | LENGTH | GONIAL<br>(GOWEN<br>& GAY ) | NEURO-<br>CYTES<br>(COMBINED<br>DATA) |                |
|    | n         | %        | n        | %        | n                               | %    |                          |                           |        |                             |                                       |                |
| 2L | 142       | 18.3±1.4 | 43       | 16.4±2.3 | 185                             | 17.7 | 38.7                     | 37.3                      | 17.8   | 37.9                        | 32.2                                  | 36.5           |
| 2R | 158       | 20.4±1.5 | 77       | 20.4±2.8 | 235                             | 22.5 |                          |                           | 20.1   |                             |                                       |                |
| 3L | 161       | 20.7±1.5 | 35       | 13.4±2.1 | 196                             | 18.8 | 39.5                     | 40.9                      | 18.3   | 42.0                        | 40.9                                  | 38.8           |
| 3R | 146       | 18.8±1.4 | 58       | 22.1±2.6 | 204                             | 19.5 |                          |                           | 23.7   |                             |                                       |                |
| 4  | 11        | 1.4±0.4  | 5        | 1.9±0.8  | 16                              | 1.5  | 1.4                      | 1.3                       | 1.4    | 1.4                         | 4.1                                   | 2.0            |
| X  | 158       | 20.4±1.5 |          |          |                                 |      | 20.4                     | 20.5                      | 18.7   | 18.7                        | 22.8                                  | 22.7           |
| Y  |           |          | 44       | 16.8±2.3 |                                 |      |                          |                           |        |                             |                                       |                |

breaks can be determined in only one class of larvae, namely X breaks in females and Y breaks in males. The breaks in autosomes are obtained from both types of larvae. Since autosomes represent approximately 80 percent of the total length of all chromosomes, the percentages of breaks for autosomes was calculated on that basis. The five long strands of the female larvae show approximately the same frequency of breaks, which also seems characteristic for the Y chromosome (KAUFMANN AND DEMEREC 1937). A comparison between the number of breaks and the number of bands in salivary chromosomes, length in salivary chromosomes, and the length in mitotic metaphases indicates a general agreement for all comparisons, although the correspondence is better in the case of salivary chromosomes.

*Distribution of breaks along chromosomes.*—The distribution of breaks along the chromosomes is given in tables 3 and 4. The positions of breaks have been determined within the limits of the divisions indicated on BRIDGES' (1935) maps. In table 3 data are arranged according to divisions, and in table 4 according to the number of breaks found in them. Cases in

TABLE 3

*Distribution of breaks along the chromosomes (sfa stands for spindle-fiber attachment region).*

| DIVISION | n  | DIVISION | n  | DIVISION | n  | DIVISION | n |
|----------|----|----------|----|----------|----|----------|---|
| 1        | 10 | 21       | 11 | 61       | 19 |          |   |
| 2        | 7  | 22       | 11 | 62       | 12 |          |   |
| 3        | 7  | 23       | 9  | 63       | 7  |          |   |
| 4        | 5  | 24       | 5  | 64       | 16 |          |   |
| 5        | 8  | 25       | 9  | 65       | 11 |          |   |
| 6        | 6  | 26       | 12 | 66       | 11 |          |   |
| 7        | 9  | 27       | 6  | 67       | 17 |          |   |
| 8        | 8  | 28       | 6  | 68       | 7  |          |   |
| 9        | 8  | 29       | 10 | 69       | 7  |          |   |
| 10       | 4  | 30       | 9  | 70       | 6  |          |   |
| 11       | 12 | 31       | 6  | 71       | 9  |          |   |
| 12       | 13 | 32       | 8  | 72       | 6  |          |   |
| 13       | 5  | 33       | 10 | 73       | 4  |          |   |
| 14       | 11 | 34       | 8  | 74       | 2  |          |   |
| 15       | 3  | 35       | 13 | 75       | 13 |          |   |
| 16       | 2  | 36       | 10 | 76       | 6  |          |   |
| 17       | 1  | 37       | 3  | 77       | 7  |          |   |
| 18       | 5  | 38       | 3  | 78       | 3  |          |   |
| 19       | 9  | 39       | 7  | 79       | 6  |          |   |
| 20       | 25 | 40       | 29 | 80       | 27 |          |   |
| sfa      |    | sfa      |    | sfa      |    | sfa      |   |
|          |    | 41       | 48 | 81       | 23 | 101      | 9 |
|          |    | 42       | 20 | 82       | 7  | 102      | 7 |
|          |    | 43       | 7  | 83       | 6  |          |   |
|          |    | 44       | 12 | 84       | 6  |          |   |
|          |    | 45       | 7  | 85       | 12 |          |   |
|          |    | 46       | 8  | 86       | 12 |          |   |
|          |    | 47       | 10 | 87       | 11 |          |   |
|          |    | 48       | 5  | 88       | 10 |          |   |
|          |    | 49       | 10 | 89       | 9  |          |   |
|          |    | 50       | 11 | 90       | 10 |          |   |
|          |    | 51       | 9  | 91       | 5  |          |   |
|          |    | 52       | 8  | 92       | 9  |          |   |
|          |    | 53       | 11 | 93       | 8  |          |   |
|          |    | 54       | 4  | 94       | 17 |          |   |
|          |    | 55       | 10 | 95       | 5  |          |   |
|          |    | 56       | 14 | 96       | 16 |          |   |
|          |    | 57       | 7  | 97       | 8  |          |   |
|          |    | 58       | 2  | 98       | 12 |          |   |
|          |    | 59       | 16 | 99       | 6  |          |   |
|          |    | 60       | 16 | 100      | 12 |          |   |

which it was impossible to determine the position of a break with reasonable accuracy have been omitted from the list.

There have been found from 1 to 48 breaks per division. The distribution of break frequencies (table 4) approximates a typical variability curve except for divisions adjacent to the spindle-fiber attachment. These di-

visions are partially or totally heterochromatic, and all except the 101 region of the fourth chromosome show high break frequencies. The values for the euchromatic divisions indicate in general a uniform distribution of breaks. The value of P for the X chromosome calculated by the  $\chi^2$  method is 0.05.

In evaluating this and the following tables, it should be kept in mind that the division by BRIDGES is not based on actual size units. The same

TABLE 4

*Distribution of breaks along the chromosomes. (Heterochromatic divisions are in italics.)*

|           |    | FEMALES ONLY:      |    |    |    |     |     |     |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|-----------|----|--------------------|----|----|----|-----|-----|-----|----|----|-----|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
|           |    | NUMBER OF BREAKS   |    |    |    |     |     |     |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    | 1                  | 2  | 3  | 4  | 5   | 6   | 7   | 8  | 9  | 10  | 11  | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
| DIVISIONS | 17 | 16                 | 15 | 10 | 4  | 6   | 2   | 5   | 7  | 1  | 14  | 11  | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|           | 58 | 38                 | 24 | 28 | 13 | 39  | 3   | 8   | 19 | 29 | 35  | 59  | 64 | 67 | 61 | 94 | 81 | 80 | 40 | 20 | 41 |    |    |    |    |    |    |    |    |
|           | 74 | 48                 | 31 | 32 | 18 | 16  | 23  | 9   | 33 | 62 | 42  | 96  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    | 37 | 52 | 27 | 63  | 25  | 21  | 44 |    | 60  | 100 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    | 45 | 54 | 30 | 79  | 36  | 22  | 49 |    | 75  |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    | 70 | 57 | 47 | 86  | 43  | 26  | 65 |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    | 73 | 76 | 51 | 89  | 68  | 34  | 66 |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    | 78 | 82 | 72 | 92  | 69  | 50  |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    | 83 | 77 | 93  | 85  | 53  |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    | 91 | 84 | 97  | 102 | 55  |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    | 95 | 88 | 101 |     | 56  |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    | 99 | 98 |     |     | 71  |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    |    |    |     |     | 87  |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    |    |    |     |     | 90  |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    | 3                  | 3  | 8  | 12 | 12  | 11  | 10  | 14 | 7  | 3   | 5   | 4  | 1  | 3  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  |
|           |    | FEMALES AND MALES: |    |    |    |     |     |     |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    | NUMBER OF BREAKS   |    |    |    |     |     |     |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    | 1                  | 2  | 3  | 4  | 5   | 6   | 7   | 8  | 9  | 10  | 11  | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
| DIVISIONS | 58 | 37                 | 54 | 24 | 27 | 39  | 32  | 23  | 29 | 21 | 26  | 35  | 56 |    | 59 | 67 | 61 | 42 | 81 | 80 | 40 | 41 |    |    |    |    |    |    |    |
|           | 74 | 38                 | 73 | 48 | 28 | 43  | 34  | 25  | 33 | 22 | 44  | 75  |    |    | 60 | 94 |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    | 78                 |    | 91 | 31 | 45  | 46  | 30  | 36 | 50 | 62  |     |    |    | 64 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    | 95 | 70 | 57  | 52  | 51  | 47 | 53 | 85  |     |    |    | 96 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    |    | 72 | 63  | 93  | 71  | 49 | 65 | 86  |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    |    | 76 | 68  | 97  | 80  | 55 | 66 | 98  |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    |    | 79 | 69  |     | 92  | 88 | 87 | 100 |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    |    | 83 | 77  |     | 101 | 90 |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    |    | 84 | 82  |     |     |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    |                    |    |    | 99 | 102 |     |     |    |    |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|           |    | 2                  | 3  | 2  | 4  | 10  | 10  | 6   | 8  | 8  | 7   | 7   | 2  | 1  | 4  | 2  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  |    |

number of breaks in two different sections, therefore, may be caused either by actual equality of size, or in case of inequality by preferred breakage in the shorter section. It is difficult to correlate the data on breaks with the size of the different sections because the maps of BRIDGES are not completely true to the actual size relations. Some chromosome regions have been relatively overstretched (for instance the middle part of 3R). Nevertheless, there is some indication of preferred breakage of some regions. As seen in table 4, the terminal sections of all chromosomes fall in break

TABLE 5  
*Break frequency in distal sections.*

| DIVISIONS | NO.<br>BREAKS | PERCENTAGES OF TOTAL<br>EUCHROMATIC LIMB |       |        | RATIO<br>BREAKS:LENGTH |
|-----------|---------------|--|-------|--------|------------------------|
|           |               | BREAKS                                   | BANDS | LENGTH |                        |
| X         | 1-2           | 17                                       | 12.78 | 9.44   | 1.35:1                 |
| 2L        | 21-22         | 22                                       | 14.10 | 9.28   | 1.52:1                 |
| 2R        | 59-60         | 32                                       | 17.11 | 10.23  | 1.67:1                 |
| 3L        | 61-62         | 31                                       | 18.34 | 11.35  | 1.61:1                 |
| 3R        | 99-100        | 18                                       | 9.94  | 7.06   | 1.40:1                 |

classes above the average value. This exceptional behavior is still more emphasized by checking the cytological length of the sections all of which are shorter than the average. Table 5 gives the values of break percentages and length relations for all terminal regions (two terminal sections in each case) as compared with the respective euchromatic chromosome limbs (sections 1-19 and the comparable ones in the autosome limbs). The ratio between break number and length which should be 1 in case of completely even distribution of breaks, however, is in all cases around 1.5. Though none of the values is statistically significant, the general trend in all chromosomes is clear and speaks in favor of preferred occurrence of breaks in the most distal chromosome parts.

The other exception is the high break number of the proximal sections except in chromosome 4. The cause of this higher break frequency may be (1) an inherent property of these regions to break more easily than euchromatic parts, or (2) a different coiling state of the chromonema as compared with that in euchromatic parts. The first possibility is based on the assumption that the salivary gland chromosomes represent completely uncoiled chromonemata. The comparison between the break numbers and the relative length of heterochromatic parts in mitotic metaphase (table 6) shows a good correspondence between break number and heterochromatin

TABLE 6  
*Comparison of frequency of breakage and mitotic length in heterochromatic regions of X and autosomes.*

|   | PERCENTAGE OF<br>BREAKS | ESTIMATED PERCENTAGE OF LENGTH<br>OF CHROMOSOME |          |
|---|-------------------------|---|----------|
|   |                         | METAPHASE                                       | SALIVARY |
| X | 15.8                    | 33.3  | 5        |
| 2 | 18.3                    | 16.6  | 5        |
| 3 | 12.5                    | 12.5  | 5        |



TABLE 7  
*Distribution of multiple breaks among the chromosome limbs.*

| NO.<br>BREAKS                               | DISTRIBU-<br>TION | NO. OF CASES |     |       | PER CENT |           |          | P         |
|---|-------------------|--------------|-----|-------|----------|-----------|----------|-----------|
|   |                   | ♀ ♀          | ♂ ♂ | TOTAL | OBSERVED |           | EXPECTED |           |
|   |                   |              |     |       | ♀ ♀      | ♀ ♀ & ♂ ♂ |          |           |
| 2   | 1, 1              | 97           | 47  | 144   | 61.78    | 65.16     | 80       | less than |
|   | 2                 | 60           | 17  | 77    | 38.22    | 34.84     | 20       | 0.01      |
| 3   | 1, 1, 1           | 2            | 6   | 8     | 7.41     | 19.51     | 48       | less than |
|   | 2, 1              | 20           | 8   | 28    | 74.07    | 68.30     | 48       | 0.01      |
|   | 3                 | 5            | —   | 5     | 18.52    | 12.19     | 4        |           |
| 4   | 2, 1, 1           | 19           | 5   | 24    | 47.50    | 48.00     | 57.6     | 0.14      |
|   | 1, 1, 1, 1        | 6            | 4   | 10    | 15.00    | 20.00     | 19.2     |           |
|   | 3, 1              | 6            | —   | 6     | 15.00    | 12.00     | 12.8     |           |
|   | 2, 2              | 9            | 1   | 10    | 22.50    | 20.00     | 9.6      |           |
|   | 4                 | —            | —   | —     | —        | —         | 0.8      |           |
| 5   | 2, 1, 1, 1        | 2            | 1   | 3     |          |           |          |           |
|   | 2, 2, 1           | 4            | 2   | 6     |          |           |          |           |
|   | 3, 2              | 6            | —   | 6     |          |           |          |           |
| 6   | 2, 2, 1, 1        | 5            | 1   | 6     |          |           |          |           |
|   | 3, 1, 1, 1        | —            | 1   | 1     |          |           |          |           |
|   | 4, 1, 1           | 1            | —   | 1     |          |           |          |           |
| 7   | 3, 2, 1, 1        | 1            | 1   | 2     |          |           |          |           |
|   | 4, 1, 1, 1        | —            | 1   | 1     |          |           |          |           |
|   | 3, 2, 2           | 1            | —   | 1     |          |           |          |           |
| 8   | 6, 2              | 1            | —   | 1     |          |           |          |           |
| Unanalyzable                                |                   | 26           | —   | 26    |          |           |          |           |
| Alterations involving<br>the 4th chromosome |                   | 15           | 3   | 18    |          |           |          |           |

length for the two autosomes, while the break number in the X heterochromatin is considerably lower. Of course, it must be realized that the determination of heterochromatic breaks may be incomplete, because, as stated above, not all intraheterochromatic rearrangements are recognizable by our method.

*Distribution of multiple breaks.*—Regarding the distribution of multiple breaks among different chromosomes, another exceptional behavior is revealed. Leaving aside chromosome 4, chance distribution of two breaks among five chromosomes with equal average break frequency (the limbs of the long autosomes have been dealt with as separate chromosomes) would

result in 20 percent of inversions (both breaks in the same chromosome) and 80 percent of translocations (both breaks in different chromosomes). In case of 3 breaks similar calculations give 48 percent of cases with 3 breaks in 3 different chromosomes, 48 percent of cases with 2 breaks in one and 1 break in another chromosome, and only 4 percent with all 3 breaks in one chromosome. These data can be derived from the formula

TABLE 8

*Distance of break points in inversions and translocations (2 break cases only).*

| REARRANGE-<br>MENTS IN<br>EUCHROMATIC<br>REGIONS | BREAK DISTANCE IN DIVISIONS |    |    |      |    |    |      |   |    |      |    |    |     |    |    |     |      |    | NO. OF<br>CASES | P  |      |
|--|-----------------------------|----|----|------|----|----|------|---|----|------|----|----|-----|----|----|-----|------|----|-----------------|----|------|
|  | 0                           | 1  | 2  | 3    | 4  | 5  | 6    | 7 | 8  | 9    | 10 | 11 | 12  | 13 | 14 | 15  | 16   | 17 |                 |    | 18   |
| Inversions<br>Observed                           | 3                           | 5  | 6  | 6    | 4  | 7  | 4    | 1 | 4  | 4    | —  | —  | 1   | —  | 2  | —   | 1    | —  | 1               | 49 | 0.61 |
| Expected   | 20                          |    |    | 16   |    |    | 8    |   |    | 3    |    |    | 2   |    |    |     |      |    |                 |    |      |
| Translocations<br>Observed                       | 7                           | 8  | 8  | 10   | 7  | 3  | 4    | 3 | 6  | 5    | 4  | 7  | 5   | 5  | 1  | 1   | 3    | 1  | —               |    |      |
| Expected   | 33                          |    |    | 17   |    |    | 22   |   |    | 12   |    |    | 4   |    |    |     |      |    |                 |    |      |
| Totals<br>Observed                               | 53                          |    |    | 33   |    |    | 30   |   |    | 15   |    |    | 6   |    |    | 137 | 0.48 |    |                 |    |      |
| Expected   | 46.2                        |    |    | 41.7 |    |    | 28.8 |   |    | 15.9 |    |    | 4.4 |    |    |     |      |    |                 |    |      |
| Totals<br>Observed                               | 10                          | 13 | 14 | 16   | 11 | 10 | 8    | 4 | 10 | 9    | 4  | 7  | 6   | 5  | 3  |     |      | 1  | 4               | 1  | 1    |
| Expected   | 64                          |    |    | 41   |    |    | 25   |   |    | 7    |    |    |     |    |    |     |      |    |                 |    |      |
| Expected   | 57.7                        |    |    | 46.9 |    |    | 25.2 |   |    | 7.1  |    |    |     |    |    |     |      |    |                 |    |      |

$(a+b+c+d+e)^n$ , a to e representing the five chromosomes in percent of the length (or break probability) of the chromosome set,  $n$  indicating the number of breaks. In our case the small autosome may be disregarded for sake of simplification and the five long strands may be taken as equal in length and breakability. The actual values vary from 17.7 to 22.5 (table 2). These differences from the assumed equality in break frequency are too small (giving about 0.1 percent deviation from the ratio 80:20 in 2-break cases) to affect the calculations. In table 7 all data are summarized according to the distribution of the breaks among the chromosomes. The first vertical column classifies breaks according to numbers observed in single nuclei, the second column subdivides these classes according to their distribution among chromosome limbs, namely 1, 1 in the 2-breaks class signifies that each of the two breaks observed in these nuclei occurred in a different limb of the chromosome set, while 2 signifies that both breaks occurred in the same limb. Other columns of that table show frequencies of various classes.

In the cases with 2, 3, and 4 breaks the data indicate that the observed distribution does not agree with expectation. In all these cases the least probable combinations occur with high frequencies. The same holds true for cases with higher break numbers for which data are not sufficient to be expressed in percentages. For instance, the only fully analyzable case with 8 breaks has an expectation frequency of less than 0.1 percent. That the observed frequencies differ significantly from the expected distribution is shown in the last columns which give the probabilities with which such distributions would be expected to occur by chance.

TABLE 9  
*Distribution of types of rearrangements.*

| NUMBER OF<br>BREAKS                 | COMBINATION<br>OF BREAKS | DOSE IN r UNITS |      |      |      |      | TOTAL         |               |
|-------------------------------------|--------------------------|-----------------|------|------|------|------|---------------|---------------|
|                                     |                          | 1000            | 2000 | 3000 | 4000 | 5000 | OB-<br>SERVED | EX-<br>PECTED |
| 4                                   | 2+2                      | —               | 3    | 17   | 11   | 9    | 40            | 9.5           |
|                                     | 4                        | —               | —    | 6    | 1    | 3    | 10            | 40.5          |
| 5                                   | 2+3                      | —               | 1    | 6    | 1    | 5    | 13            |               |
|                                     | 5                        | —               | —    | 1    | —    | 1    | 2             |               |
| 6                                   | 2+2+2                    | —               | —    | 2    | —    | 1    | 3             |               |
|                                     | 3+3                      | —               | —    | 1    | —    | —    | 1             |               |
|                                     | 2+4                      | —               | —    | 2    | 1    | 1    | 4             |               |
|                                     | 6                        | —               | —    | —    | —    | —    | —             |               |
| Higher numbers and<br>unclear cases |                          | 1               | 1    | 9    | 5    | 10   | 26            |               |

*Distance between breakage points.*—In table 8 data are arranged to show the length of inversions and the distance between breakage points in translocations expressed as the difference of length from the breakage point to the spindle fiber attachment. In this summary, two-break cases only have been included, as it is possible that higher breaks within one sperm would obscure the results. Cases in which one break occurred within the heterochromatic region were omitted since the possibility is not excluded that the true relationship between lengths in heterochromatin and euchromatin might be different than expressed in salivary chromosomes. The top line in table 8 indicates classes expressed in divisions of BRIDGES' (1935) map of distance between two breaks. In the other columns are given the observed frequencies for each class and for groups of four classes together with the expected frequencies for four class groups. At the lower end of the table, classes are summarized in groups of five in order to increase the number of cases in each group. Probabilities (P) calculated by the  $\chi^2$  method show a rather good correspondence between observed and expected values, indicating that two breaks in euchromatic regions occur at random, namely independently of each other.

*Distribution of types of rearrangements.*—If random combination takes place between all break ends, some of the rearrangements will simulate simultaneously occurring independent rearrangements. For instance, four breaks will give a certain number of cases with two independent inversions or translocations among the majority which represents complicated exchanges involving all eight break ends. The ratio between both these types (2+2 and 4) is different with different distribution of the breaks among the chromosomes, due to differences in the number of viable recombinations. If the breaks have occurred in four different limbs (1, 1, 1, 1), the ratio 2+2:4 will be 3:6. In 2, 1, 1-cases it will be 3:14, and in the other combinations (2, 2; 3, 1; and 4) 3:22. A test of our data (table 9) shows that the actual relation between single complicated and multiple independent rearrangements is just the reverse. In all break classes the cases with several independent rearrangements, each with 2 or 3 breaks only, overweigh by far. No clear 6-break case has been found representing a simultaneous rearrangement among all break ends. In table 9 the expected values are calculated for 4-break distributions only, since the data in other cases are not large enough to warrant such calculations.

*Rearrangements and contact points.*—In order to consider the mechanism through which various observed rearrangements may be accomplished,

TABLE 10  
*Analysis of the rearrangements in terms of the "contact hypothesis."*

| DOSAGE IN<br>r UNITS | NUMBER OF "CONTACT POINTS" REQUIRED |    |    |       |   |   |   |
|----------------------|-------------------------------------|----|----|-------|---|---|---|
|                      | 2                                   | 3  | 4  | 5     | 6 | 7 | 8 |
| 1000                 | 8                                   | 3  | —  | —     | — | — | — |
| 2000                 | 23                                  | 6  | —  | —     | — | — | — |
| 3000                 | 171                                 | 27 | 8  | 1     | — | — | 1 |
| 4000                 | 67                                  | 9  | 2  | —     | — | — | — |
| 5000                 | 77                                  | 19 | 6  | 1     | 1 | — | — |
| Totals               | 346                                 | 64 | 16 | 2     | 1 | — | 1 |
|                      | 80.5%                               |    |    | 19.5% |   |   |   |

it is helpful to determine the number of strands which should be in contact at one point, provided such a condition is essential that recombinations between strands in contact may occur. In table 10 data are summarized to show the number of contact points. The first line of that table gives classes indicating the number of strands required to be in contact at one point in order that certain observed rearrangements may be accomplished. In the other part of the table observed frequencies for different classes are listed. It is evident that in 19.5 percent of the cases more than two strands would

be required to be in contact and one case has been analyzed in which a contact of eight strands would be required to furnish the observed rearrangement.

#### DISCUSSION

The possible mechanisms of the origin of induced chromosomal rearrangements have been discussed by several authors, notably SEREBROVSKY (1929), and STADLER (1932).

Chromosomal rearrangements depend on separation of preexisting genic connections and reunion in new combinations. A priori two alternative explanations are possible. (1) Free breakages are independently induced and reunion in twos follows later (STADLER). In this event different consequences are to be expected dependent on whether the process of reunion is limited in space and time or not. If it is, then the results will not differ much from those expected on the basis of the second possibility, which is that (2) breakage and reunion are part of one process and occur simultaneously. Here again two rather divergent mechanisms can be, and have been, proposed. In the first place, (2a) external attachment may precede both breakage and reunion which then will take place in mechanical ways only at regions of original contact (SEREBROVSKY). On the other hand, (2b) broken ends independently induced as in (1) may, by nature of their "unsaturated" attraction forces, cause the rupture of intact chromosomes with which they lie in contact. While the mechanism (2a) would work at microscopic dimensions, the latter could be effective at molecular distances only, that is in places where the chromosomes are as closely apposed as the homologues are in meiotic synapsis.

X-ray treatment would act in (1) and (2b) by increasing the breakage number and thus augmenting the number of rearrangements. In (2a) it would lead to a change of the general, perhaps colloidal, state of the nucleus by which the chromosomes become "sticky." For this possibility there is a remarkable parallel in the case of homozygous sticky plants in *Zea mays*. BEADLE (1937) has shown that in the progeny of these, the normal mutation rate is raised approximately ten times as compared with heterozygous, or wild type plants. This increase is observable in both chromosomal rearrangements as well as in apparent gene mutations. The cause in this case is clear from direct observations. The chromosome substance is changed by the sticky gene in such a way as to become gluey, thus giving all kinds of attachments. That similar changes can be the result of X-ray treatment has recently been shown by WHITE (1937).

The first point in considering the different possibilities cited above, is to determine whether random combination between breakage ends occurs or not. The distribution of the breakages seems to be random among and

along the chromosomes with two possible exceptions. (1) The behavior of heterochromatic parts is still open to discussion. KAUFMANN and DEMEREC (1937) from their data on break frequencies in Y sperms, have reached the conclusion that there is in all chromosomes including the Y a breakage frequency directly proportional to their metaphase length. From this they further conclude that the chromonema length is directly proportional to metaphase length, as suggested by MULLER and PAINTER (1932); moreover that the Y therefore is of the same general structure as euchromatic chromosomes, and that direct proportionality exists between chromonema length and breakage numbers in all chromosome parts. They are forced to consider the relative length of hetero- and euchromatic regions in salivary gland chromosomes to be secondarily disturbed by differential uncoiling, the heterochromatic parts and the Y chromosome remaining in a state of submicroscopic folding. Cytological tests are not yet available. They might be obtained, however, from observations in polarized light. The opposite hypothesis put forward by MULLER and GERSHENSON (1935) assumed that the salivary gland chromosomes represent the real proportions of chromonema length of the different sections, and that in mitosis the relative length of eu- and heterochromatin is changed due to non-spiralization of the heterochromatin. This hypothesis seems to have found support by the genetic evidence obtained by MULLER and co-workers (MULLER, RAFFEL, GERSHENSON and PROKOFYEVA-BELGOVSKAYA 1937; MULLER, PROKOFYEVA-BELGOVSKAYA and RAFFEL 1938) on the differential effect of duplications and corresponding deficiencies on certain parts of the inert region of the X chromosome in its metaphase length. The so-called block "A," though a small part of the heterochromatin only, seems to be mainly responsible for the length of the inert region in mitosis, while on the basis of KAUFMANN and DEMEREC's assumption metaphase length should be proportionally affected by these duplications.

Our data pertaining to this problem are summarized in table 6, from which it is evident that, for autosomes, there is a close agreement between observed values for breaks and the length of heterochromatic regions as measured in metaphase chromosomes. However, the break value for the X chromosome is intermediate between that expected from metaphase length and that expected from salivary length. It has been pointed out that inversions and translocations with both breaks in the heterochromatic region cannot be detected by the method used in this study. If metaphase length is assumed to represent equivalent chromonema lengths in all regions, then the length of the heterochromatin of X should be equivalent to about nine euchromatin divisions. The data shown in table 8 indicate that about 90 percent of inversions occur within a distance of nine divisions and from the data for 2-break cases shown in table 7, it may be inferred

that about 35 percent of breaks are connected with such inversions. By taking into account all these factors, an adjustment for undetectable inversions can be made which brings the figure up from 15.8 to about 22.0 percent. The adjusted figure is still appreciably below the expected 33.3 percent, although an additional correction for undetectable heterochromatic translocations would bring it still closer to the expected value. The significance of this discrepancy may possibly be determined through additional data from experiments which are now in progress. It seems advisable to refrain from passing judgment on this question until the additional evidence becomes available and for the present to consider both possibilities, namely that (1) salivary chromosomes give a true picture of the actual length of the heterochromatic region and (2) that the true picture is evident from metaphase chromosomes.

The other possible exception to random distribution of breaks is the break frequency of distal ends, which is slightly, but consistently, higher than that of the other euchromatic sections.

The interpretation of these results must be different according to the different possible mechanisms. If breaks are produced independently, then we would observe only a fraction of them, the realized breaks, while others must have disappeared by fusion of the original breakage ends. The observed differences from random distribution, therefore, may be due to both higher frequency of induced breaks and lower probability of disappeared breaks.

The second point concerning the arrangement of chromosomes in the sperm head is whether random exchange occurs between all chromosome parts. Several facts are revealed from our data. First, the different types of break combinations show differences from expectancy. Accumulation of breaks within one chromosome is evident for 2-break cases and highly probable in case of more breaks (table 7). Secondly, as far as analyzable (table 9), four and more breaks very rarely lead to a simultaneous exchange among all altered chromosomes, but are dissolvable into two or more independent rearrangements each involving fewer breakage points. This result contradicts the data collected by DUBININ and KHVOSTOVA (1935), but as they have dealt with a selected group of aberrations their results do not seriously affect our conclusion.

These facts taken together indicate that the combination of break loci is not at random but is restricted. No attempt will be made to define more precisely what spatial arrangement is responsible for the different phenomena. But the general cause of them must be that rearrangements are limited in space and time.

No sign of a very constant arrangement of the chromosomes is obvious. PATTERSON, STONE, BEDICHEK and SUCHE (1934) have obtained results

from which they arrived at a very special picture of the architecture of the sperm-head. All ends, proximal and distal ones, are assumed to be polarized towards the same part of the nucleus. This picture was derived from the fact that translocation breakage occurred preferably in the spindle fiber regions and in the distal ends. Our data are not in accord with theirs. The reason may partly be that a number of translocations with both breaks in the chromocentral parts escaped us due to the chosen method. The apparent increase of breaks in the distal ends in PATTERSON's material, much higher than that we have found, may partly be a consequence of the differences between the cytological and the genetical maps.

From the results on the location of breakage points it is not possible to decide between the different schemes for the origin of rearrangements. Our data indicate, however, that breakage and recombination must be local phenomena.

KIRSSANOW (1937), from a study of the percentage relation between reciprocal and triple translocations, believes he has obtained decisive evidence against the break-hypothesis and for the so-called crossing-over hypothesis, our alternative 2. In his calculations, however, he reckons only with the possibility of free combination in case of induced free break ends. In light of the results presented in the present paper, his positive decision in favor of the contact hypothesis is unwarranted.

From the types of chromosomal rearrangements, conclusions have been drawn as to the mechanism of their origin. One of the most obvious points is that single breakage rearrangements have been found only very rarely, none in the present study. Even if the distal ends of chromosomes are for internal reasons incapable of accepting free fragments, terminal deficiencies should occur more often. Their absence in most cases has led several authors, especially MULLER and his associates to doubt the very existence of 1-breakage cases, that is, terminal deficiencies. MULLER believes that, the mechanism of origin of rearrangements being somehow related to the crossing-over process, two breaks is the minimum number inducible. This conclusion, however, has not been proved. Even if it were assumed that there are no terminal deficiencies among the stocks, and that all described cases, especially those of DEMEREC and HOOVER (1936), may be regarded as representing 2-breakage cases with the distal break very near the tip, the possibility remains that the absence of such deficiencies may be due to elimination rather than to non-occurrence. This elimination of course is not due in all cases to hypoploidy but may be a consequence of the behavior of broken ends of chromosomes, such as CARLSON (1938) has described in irradiated neuroblast cells of grasshoppers. If new free ends are unstable and tend to reattach themselves to other similar free ends, then the two chromatids of the same chromosome with the original terminal deficiency will unite and thus form a bridge at next anaphase. This bridge



will break again, and there is no reason to believe the new connection between both ends will be weaker than other places, so that the next break will take place by chance leading to two broken chromosomes of different lengths. This process must recur at many or all following divisions, thus gradually increasing the differences in genic content of the daughter cells. Thus, inviable combinations will necessarily result and in the end the r-breakage cases will be eliminated. Therefore, their absence does not disprove possibility (1) of their origin. On the other hand, the multiple cases are rather difficult to explain on the basis of possibility (2b). At any given point a close contact between two chromosomes may reasonably be assumed but not between more. As indicated in table 10, rearrangements requiring more than two contact points constitute about 20 percent of the total. One case has been analyzed requiring contact of eight strands. On the other side, DUBININ and KHVOSTOVA (1935) have offered another variation of the contact hypothesis by suggesting that multiple rearrangements take place in parts of the nucleus where the presumptive breakage regions have become entangled into a knot. As in this case knot-formation is independent of the following irradiation one would expect that the same relative amount of multiple breakage-cases would occur with all dosages. Our data, although not extensive enough to warrant a general conclusion, suggest, however, that multiple breaks occur more frequently at higher dosages.

Important for further considerations is the relation between X-ray dosage and percentage of aberrations. For the different possibilities of the origin of rearrangements, different curves might be expected. A linear relation would result in (2b) if the induced breaks are a direct consequence of the ionization. An exponential curve will result from a mechanism suggested by STADLER at least for those dosages which do not induce more than two breaks in one sperm. Higher breakage numbers and limited combination possibilities will lead to more complicated relations. According to possibility (2a) probably an S-shaped curve as typical for chemical reactions might be expected.

Table 1 and figures 1 and 2 suggest that a direct proportionality does not exist between dosage and the number of sperms with changes, the number of aberrations, and the number of individual breakages. Suggestive is the strong increase between 2000 and 3000 r units. The trend of the resulting curve is not determinable owing to the large standard errors.

These results contradict certain published data. HEPTNER and DEMIDOVA (1936) believe that from 1000 to 4000 r units there exists a direct linear relation for point mutations, deficiencies and other rearrangements as well. The separation between the different types and their grouping, however, seems not clear thus making their data unreliable. Of a different nature is the direct proportionality found by KHVOSTOVA and GAVRILOVA

(1935) for translocations showing position effect for  $ci^+$ . This result is significant but does not necessarily mean that the translocations result from a mechanism elaborated under (2b) but can well be interpreted by the hypothesis of STADLER (1). In these special cases two breakages of different probabilities are involved. One breakage necessarily must be next to  $ci^+$ , the other, however, may be anywhere along the euchromatic limbs of the other chromosomes or in the heterochromatin of X or in the Y. The probability for this second type of breakage, therefore, is much higher, so that practically in every case when a break will have occurred near  $ci$  there is a second one available for recombination. Nearly every breakage at  $ci$  then will lead to a rearrangement. As in these cases a direct proportionality was observed this suggests that either the rearrangement as a whole or the independent break is directly induced. The other results presented here do not favor the first explanation and, therefore, justify the following conclusions, that (1) breakages are independently induced by the ionization in a way comparable to point mutations, as assumed by STADLER, and that (2) recombination between free breakage ends occurs in a limited space and time.

One possible objection to these conclusions is the fact that small deficiencies involving one band are rather frequent (SLIZYNSKA 1938). They would have to be interpreted as representing two breaks occurring very close together, but their actual number seems to be much higher than that predictable on the basis of chance occurrence of such breakages. For this reason the other possibility must be held open, namely that short deficiencies have an origin not related to that of other chromosomal aberrations but more similar to that of point mutations. The frequent occurrence of induced chromosomal aberrations with lethal effects might then be due to one point of the rearrangement representing a true break, the other one being an independent deficiency, as has been suggested by DEMEREC (1937).

Data obtained by DEMEREC (1938) indicate that Oregon-R stock, the same one which was used in these experiments, has a low induced mutation rate when compared with Swedish-b stock and with the other material for which data are published. In the experiments referred to, 1000 r-units induced  $1.30 \pm 0.24$  sex linked lethals, 3000 gave  $2.15 \pm 0.45$ , 4000 produced  $3.08 \pm 0.47$ , and 5000 r-units induced  $5.43 \pm 0.76$  lethals. When these genetic results, showing the relation between dosage and the frequency of induced X chromosome lethals, are compared with the cytological results given in table 1 and figure 1, showing the relationship between dosage and the frequency of sperms with chromosomal aberrations, it can be seen that cytological values for 3000 to 5000 dosages are slightly higher than genetic values, while the value for 1000 r-units is appreciably lower. The figures of table 1 represent aberrations observed in all chromosomes

obtain values for the X chromosome only, these figures should be reduced to 18.7 percent. Recalculated figures are 0.68, 1.62, 4.98, 5.57, 7.50 percent for 1000, 2000, 3000, 4000, and 5000 r-units respectively. It is known that not all chromosomal aberrations are connected with lethals and that not all lethals are connected with chromosomal aberrations. Evidence is also available that the incidence of aberrations is disproportionally higher among lethals induced by stronger treatment (DEMEREK 1937). It is of interest to note that a similar relationship between aberrations and lethals is evident here. At 1000 r-units the value for lethals is higher than the value for aberrations, while at higher dosages the reverse is true. This relationship could be explained by assuming that a similar mechanism is responsible for both lethals and aberrations, namely that a portion of the lethals originate at a breakage point. If chromosomal rearrangements are formed through breakages and subsequent reattachments, then at low dosages, where breaks are rare and occur mostly singly in individual nuclei, a broken chromosome would be restored by a reattachment, while at higher dosages, which induce multiple breaks in nuclei, such reattachments may produce chromosomal rearrangements. Thus it is to be expected that low dosages would give a higher proportion of lethals not connected with chromosomal aberrations and therefore that the relationship between values for lethals and values for aberrations would be different at low and at high dosages.

The data shown in table 8 indicate that distances between two breaks are distributed at random, namely that two breaks occurring in the same chromosome are independent of each other. Since it is definitely known that in case of crossing over the position of the second break is influenced by the position of the first break, the present results suggest that a different mechanism is responsible for reattachments connected with crossing over than for those producing induced inversions and translocations.

#### SUMMARY

Chromosomal rearrangements were studied by salivary gland chromosome analysis of 1765  $F_1$  larvae obtained from untreated females mated to X-rayed males. A total of 1038 breaks were plotted in euchromatic regions within the limits of the single divisions of BRIDGES' (1935) maps.

The data indicate a significant deviation from the straight line proportionality between dosage and frequency of breaks.

Distribution of breaks among chromosomes of comparable lengths is at random.

In general, the distribution of breaks is at random within euchromatic sections of chromosomes, with the possible exception of distal regions where breaks tend to be slightly more frequent. In heterochromatic regions, breaks are more frequent than in euchromatic regions for similar

Two breaks occurring in euchromatic regions are independent of each other. In case of multiple breaks a tendency is noticeable for them not to be distributed at random among chromosomes but to accumulate within chromosomes.

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# ON THE INHERITANCE AND EXPRESSION OF A MOTTLED-EYED MUTANT IN *DROSOPHILA MELANOGASTER*<sup>1</sup>

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Received July 28, 1938

## INTRODUCTION

MOTTLED-EYED flies have been reported on various occasions by numerous investigators. The following paper presents further data on the inheritance and expression of a mottled-eyed mutant of *Drosophila melanogaster*. The mutant first appeared in a progeny of flies which had been subjected to supersonic vibrations in an experiment conducted by HERSH, KARRER and LOOMIS (1930), in an attempt to induce mutation. In this experiment there appeared five mottled-eyed males in the progeny of 26,135 flies, resulting from matings of treated cut forked Bar males with virgin double-yellow females with attached X chromosomes. This fact suggested sex linkage. The mottled males were bred to the double-yellow sisters and the flies in the F<sub>1</sub> were normal in each case. Upon allowing the F<sub>1</sub> to interbreed some mottled flies of both sexes appeared in the F<sub>2</sub>. The character was then carried along with a greater or smaller number occurring in each generation.

The mutant was later crossed with a vermilion stock by DR. W. P. SPENCER thus producing a stock of vermilion mottled-eyed flies. Such was the nature of the stock at the beginning of this investigation.

The mutant shows a pronounced dark mottling of the eyes and was illustrated by SURRARRER (1935). These illustrations show variations from almost complete dark pigmentation to nearly entire removal. At temperatures of 25°C and above complete removal of the dark pigmentation results. Not only is there variation in the magnitude of the effect, but also a considerable variation in the compactness of the darkened area.

Because of this variability and frequent lack of the character expression under ordinary culture conditions, it was necessary to find control methods for production. The author found (1935) that the character was expressed at temperatures of 20°C or below. It was further found that the temperature-effective period was in the pupa stage and that the percentage of the area of the eye affected was inversely proportional to the developmental temperature.

If the main problem of genetics and development is the determination

<sup>1</sup> Submitted to the Division of Biology of Western Reserve University in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Sincere appreciation is here expressed to Dr. A. H. Hersh of Western Reserve University under whose direction the work was accomplished.

of the intermediate action between the genes at the beginning and the external characters at the end of development, such a mutant presents rather unique material for a study of genic expression. With a controlled procedure for the expression of the character there was furnished the necessary information for further study of the genetics of the mutant.

#### PROCEDURE AND RESULTS OF GENETICAL ANALYSIS

The flies were cultured in large stock bottles on 1 percent agar agar banana media. They were placed at 27°C for increased egg production and rapid larval development. Before the beginning of pupation the adults were removed and the cultures placed at 18°C for further development. Mottling may be prevented from developing in the adults by subjecting their pupae to temperatures of 25°C or above. The question immediately arises, will such red individuals, thus produced, have the same hereditary qualities as the mottled adults, if the pupae are subjected to the same environmental conditions.

Mass cultures of the mottled mutant were allowed to develop at 27°C. Before the time of pupation the adults were removed, and part of the cultures placed at 18°C for further development. Upon emergence two mass cultures were made up from the phenotypically non-mottled adults which had been produced at 27°C, and two like cultures from the above mentioned phenotypically mottled adults produced at 18°C. The offspring thus produced, in accord with general expectation, showed no appreciable difference in character expression.

The localization of the mottled gene<sup>2</sup> in its respective chromosome was carried out with a physiologically balanced stock *S/Cy D/C III X*. Mottled virgin flies were crossed to *S/Cy D/C III X* males. The *F*<sub>1</sub> without exception was non-mottled. This confirmed the recessive nature of the mutant and further proved the character was not sex linked. The Star Dichaete *F*<sub>1</sub> males were crossed back to mottled females, and there appeared in the progeny Star mottled, but no Dichaete mottled flies. This showed that mottled is in the third chromosome. This fact is further confirmed by crossover data.

Selected Dichaete males from the above described cross were mated to virgin mottled flies. The following results were obtained from the first backcross.

|                                   |         |        |
|-----------------------------------|---------|--------|
| <i>D/mot</i> ♂ × <i>mot/mot</i> ♀ |         |        |
| Dichaete                          | mottled | Normal |
| 123                               | 119     | 10     |

The appearance of normal in the offspring was assumed to be due to modifiers. This is partly proved by the results of the next back-cross.

\* This gene has been given the symbol *mot*<sup>23</sup> but for the sake of simplicity is referred to throughout this paper as *mot*.

|                                     |         |        |
|-------------------------------------|---------|--------|
| <i>D/mot</i> ♂ × <i>mot/mot</i> ♀ ♀ |         |        |
| Dichaete                            | mottled | Normal |
| 280                                 | 251     | 0      |

Assuming mottled to be a typical Mendelian recessive, the above data, even though small, fall within acceptable deviation.

|             |          |         |
|-------------|----------|---------|
|             | Dichaete | mottled |
| Observed    | 280      | 251     |
| Expectation | 265.5    | 265.5   |
| Difference  | 14.5     | 14.5    |

From the formula  $\sigma = \sqrt{qnp}$  it is found that the probable error is within acceptable limits.

In the isolation of the Dichaete flies for the above crosses it was noticed that Star flies were usually only slightly mottled. The influence of Star was checked by producing mottled Star flies and then backcrossing to mottled. The offspring from such a cross were carefully checked. In order to illustrate the comparative degree of mottling the following method of estimation was used. When  $\frac{3}{4}$  of the area of the eye was estimated to be pigmented, it was recorded as 4, when approximately  $\frac{1}{2}$  as 3, less than  $\frac{1}{2}$  as 2, and when only slightly pigmented as 1.

|              |   |    |   |   |  |   |   |    |    |
|--------------|---|----|---|---|--|---|---|----|----|
|              | <i>S/+mot/mot</i> ♀ ♀ × <i>+/+mot/mot</i> ♂ ♂ |    |   |   |  | <i>S/+mot/mot</i> ♂ ♂ × <i>+/+mot/mot</i> ♀ ♀ |   |    |    |
|              | <i>S/+mot/mot</i>                             |    |   |   |  | <i>+/+mot/mot</i>                             |   |    |    |
| Area mottled | 1   | 2  | 3 | 4 |  | 1   | 2 | 3  | 4  |
| Individuals  | 42  | 11 | 3 | 0 |  | 1   | 6 | 29 | 41 |

From the above data, it seems that Star acts as an inhibitor to the area of the eye effected by mottled.

After the homozygosity was such that the offspring from *mot/mot* ♀ ♀ × *D/mot* ♂ ♂ were either mottled or Dichaete, reciprocal crosses were made to determine the crossover value between mottled and Dichaete. Mottled males were crossed to *D/mot* females, resulting in offspring as illustrated in table 1.

TABLE 1  
Crossover data involving Dichaete and mottled  
*D/mot* ♀ ♀ × *mot/mot* ♂ ♂.

| DICHAETE | MOTTLED | NON-DICHAETE<br>NON-MOTTLED | DICHAETE MOTTLED |
|----------|---------|-----------------------------|------------------|
| 802      | 666     | 50                          | 35 (Total 1555)  |

From the data it is evident that Dichaete flies are apparently more viable than mottled ones. It was expected that the crossover columns

III and IV would more closely approach each other; yet it would be logical to assume that the phenotypically normal flies would be more viable than the mutants. According to the foregoing data the crossover value of mottled and Dichaete is 5.46 percent. From this result mottled is either in close proximity to the spindle fiber or is in the approximate position of rose.

The cross  $D/mot \times mot/mot$  used in the attempt to determine the position of mottled from Dichaete was allowed to inter-breed during the summer of 1936 from June until August. At the end of the three months' period virgin mottled crossed to Dichaete males gave as in the previous work either Dichaete or mottled offspring in accord with expectation. Dichaete virgin flies from the above lines were chosen and crossed to the mottled males from the same strain,  $D/mot \text{ } \varnothing \text{ } \varnothing \times mot/mot \text{ } \sigma \text{ } \sigma$  resulting in some mottled Dichaete flies. From such crosses the mottled Dichaete male and female flies were carefully selected, and each sex placed in shell vials and crossed back to the mottled line. In this way a mottled Dichaete strain was easily maintained by crossing Dichaete mottled males to mottled females with constant selection of the Dichaete mottled males.

A Stubble line of *D. melanogaster* was obtained from Pasadena in 1935 with the third chromosome constitution  $S/C_3 \text{ } 13a$ . These flies were crossed to mottled flies which had been inbred with the Dichaete line previously mentioned.  $(+/+ \text{ } +/+ \text{ } Sb/C_3 \text{ } 13a \text{ } +/+ ) \times (+/+ \text{ } +/+ \text{ } mot/mot \text{ } +/+ ) \rightarrow +/+ \text{ } +/+ \text{ } Sb/mot \text{ } +/+$  and  $+/+ \text{ } +/+ \text{ } mot/C_3 \text{ } 13a \text{ } +/+$ . The Stubble males were carefully selected and crossed back to the previously mentioned mottled flies. The  $F_1$  generation showed a predominance of Stubble and thus to increase the homozygosity of the lines the Stubble males were chosen and crossed back to the mottled females of the previously mentioned line for seven generations. In all cases Stubble flies outnumbered the expectation of a 1:1 ratio and in no case did the non-Stubble exceed the fifth backcross in which the non-Stubble flies comprised 45.7 percent of the progeny.

The Stubble males of the seventh backcross were carefully selected and crossed to virgin Dichaete mottled females.

$$Sb/mot \text{ } \sigma \text{ } \sigma \times D \text{ } mot/mot \text{ } \varnothing \text{ } \varnothing$$

Ten such crosses were made up on 1 percent agar banana media in large culture bottles and incubated as previously described for character expression. The  $D \text{ } mot/+ \text{ } + Sb$  females thus produced were crossed to  $mot/mot$  males. Ten of these crosses were made up with approximately 10 females and 20 males in each bottle. At the end of three days the adults were removed and placed in new culture bottles for an additional three day egg laying period, and incubated as above.

The flies from the previously mentioned crosses were carefully checked



under a Spencer binocular with a 23× objective and 9× ocular combination. The results of the cross are listed in table 2.

TABLE 2  
*Crossover data involving Dichaete, mottled and Stubble.*

| NON-CROSSEOVERS  |     |      |      | CROSSEOVERS   |    |    |    |    |    |     |     |     |   |     |     |
|--|-----|------|------|---|----|----|----|----|----|-----|-----|-----|---|-----|-----|
| $\frac{D \text{ } mot \text{ } +}{+ \text{ } + \text{ } Sb}$ |     |      |      | $\frac{\begin{matrix} \text{♀} \text{ } \text{♀} \\ + \text{ } + \text{ } Sb \end{matrix} \times \begin{matrix} + \text{ } mot \text{ } + \\ + \text{ } mot \text{ } + \end{matrix}}{\text{♂} \text{ } \text{♂}}$ |    |    |    |    |    |     |     |     |   |     |     |
| $\frac{D \text{ } mot \text{ } +}{+ \text{ } + \text{ } Sb}$ |     |      |      | $\frac{D(1) \text{ } mot(2) \text{ } +}{+ \text{ } + \text{ } Sb}$  |    |    |    |    |    |     |     |     |   |     |     |
|  |     |      |      | 1   |    | 1  |    | 2  |    | 2   |     | 1:2 |   | 1:2 |     |
| ♂  | ♀   | ♂    | ♀    | ♂   | ♀  | ♂  | ♀  | ♂  | ♀  | ♂   | ♀   | ♂   | ♀ | ♂   | ♀   |
| 343  | 418 | 1339 | 1589 | 81  | 66 | 39 | 54 | 22 | 27 | 109 | 120 | 3   | 4 | 100 | 128 |
| Total 4442   |     |      |      |   |    |    |    |    |    |     |     |     |   |     |     |

From table 2 it is evident that all of the expected flies occurred in the population. It is further evident that in all cases except the Dichaete Stubble flies the viability of the females is slightly above that of the males. This is, however, probably not significant, and somewhat in accord with the expectation.

The distorted ratio of the non-crossover flies (761 Dichaete mottled to 2929 Stubble) is probably significant. The expected ratio is 1:1 and the result is 1:3.85. Because of the low viability of the Dichaete mottled flies when in competition with Stubble, the Dichaete mottled crossover percent has not been considered of value.

The crossover value of Stubble and mottled as determined from table 2 is as follows:

|          |            |           |                          |           |
|----------|------------|-----------|--------------------------|-----------|
| <i>D</i> | <i>mot</i> | <i>Sb</i> | } Dichaete <i>mot Sb</i> | 49        |
| +        | <i>mot</i> | +         |                          |           |
| +        | +          | +         | } Normal                 | 229       |
| +        | <i>mot</i> | +         |                          |           |
| <i>D</i> | +          | +         | } Dichaete               | 228       |
| +        | <i>mot</i> | +         |                          |           |
| +        | <i>mot</i> | <i>Sb</i> | } mottled Stubble        | 7         |
| +        | <i>mot</i> | +         |                          |           |
|          |            |           |                          | <hr/> 513 |

$$513 \div 4442 = 11.54\%$$

To further confirm the position of mottled by the crossover method the *Sb/mot* males were again selected and crossed back to mottled females to further increase homozygosity. The viability of Stubble was again, as in all previous cases, above that of mottled.

Mottled males were then crossed back to Stubble heterozygous mottled females.

$$Sb/mot \text{ } \text{♀} \text{ } \text{♀} \times mot/mot \text{ } \text{♂} \text{ } \text{♂}$$

Seven such crosses were made up on 1 percent agar banana media in large stock bottles. Ten females with about twenty males were placed in each bottle and incubated as in previous experiments.

TABLE 3  
*Crossover data involving mottled and Stubble*  
*Sb/mot ♀ × mot/mot ♂*.

| NON-CROSSEOVERS |     |         |     | CROSSEOVERS     |    |        |    |
|-----------------|-----|---------|-----|-----------------|----|--------|----|
| MOTTLED         |     | STUBBLE |     | MOTTLED STUBBLE |    | NORMAL |    |
| ♂               | ♀   | ♂       | ♀   | ♂               | ♀  | ♂      | ♀  |
| Totals 178      | 195 | 546     | 580 | 26              | 30 | 61     | 93 |

From the data in table 3 there is a total of 1709 flies with 210 crossovers between *Sb* and *mot*.

$$210 \div 1709 = 12.2\% \text{ crossover value}$$

This crossover value compares with the previous mottled, Stubble crossover value within 1.66 units.

The crossover value as determined when Stubble heterozygous mottled and Dichaete mottled were mated was as follows:

|          |            |           |                    |           |
|----------|------------|-----------|--------------------|-----------|
| <i>D</i> | +          | <i>Sb</i> | } Dichaete Stubble | 147       |
| +        | <i>mot</i> | +         |                    |           |
| +        | <i>mot</i> | +         | } mottled          | 93        |
| +        | <i>mot</i> | +         |                    |           |
| <i>D</i> | +          | +         | } Dichaete         | 228       |
| +        | <i>mot</i> | +         |                    |           |
| +        | <i>mot</i> | <i>Sb</i> | } mottled Stubble  | 7         |
| +        | <i>mot</i> | +         |                    |           |
|          |            |           |                    | <hr/> 475 |

$$475 \div 4442 = 10.6\%$$

Thus mottled is placed between 47.66 and 51. However, as previously stated, the crossover value of Dichaete and mottled when obtained in competition with Stubble is probably not valid. The Stubble mottled crossover value, on the other hand is apparently valid. Thus the mottled Stubble crossover value when determined independently of Dichaete is 12.2; while that obtained in combination with Dichaete is 11.54. The combined data give a value of 11.87.

Even though all the crossover data do not exactly conform, it is evident that mottled is very close to the spindle fibre of chromosome three. The spindle attachment has been placed on the standard map at 46. From the above, the data indicate mottled to be between 45.86 and 46.33; the mean of which is 46.08.

Thus from the foregoing data the mutant is definitely placed on chromosome three as shown by a dominant physiologically balanced stock and further confirmed by the above linkage data. The three point linkage data place the character in close proximity to the spindle fiber attachment. It was found by GLASS (1933) in X-ray induced dominant mosaic eye-color mutants that chromosome breaks were at or very near the locus involved and further that in chromosome II and III frequent breaks occur in the vicinity of the spindle fiber. SCHULTZ (1936) believes that a translocation of inert regions from the spindle attachment or other loci to active regions is the probable reason for variegation.

Whether the character involves a visible translocation or inversion will only be definitely determined by cytological salivary analysis.

#### THE TEMPERATURE EFFECTIVE PERIOD AND ACTUAL EXPRESSION

The author (1935) showed that the temperature effective period of the mottled mutant was during the first half of the time interval between pupation and emergence. A further analysis of the development was undertaken in an attempt to more accurately place the temperature effective period of the character.

#### PROCEDURE AND RESULTS

Large families of flies were made up on 1 percent agar agar banana media in straight  $2\frac{3}{8} \times 3\frac{3}{4}$  inch Stender glasses. Such containers were used to facilitate recovery of pupae. These cultures were maintained at 27°C. The adults were removed upon the appearance of the larvae and the containers carefully watched until pupae development approached a peak. All existing cases were then removed and from that time pupae were taken out at hourly intervals.

This experiment was carried out in an underground room where temperature fluctuation was rather slight. It was thus possible to maintain a

temperature under a goose neck lamp of approximately 27°C during the removal of the pupae. The pupae were carefully taken out by the use of a wire loop and placed on a narrow strip of paper toweling which extended down into approximately 3 cc. of water in the bottom of the test tube. The tubes were then plugged with cotton in the usual manner. In this way a rather constant humidity was maintained which seemed to satisfy conditions for future emergence. The tubes were numbered and immediately returned to the 27°C incubator. Each tube then remained at 27°C for a definite time interval ranging from 24 to 39 hours, preliminary experiments having revealed the effective period to be within these limits. The data recorded in table 4 were obtained from two sets of nine families each. The data from each set overlapped the other as to time interval. From the two sets of data a total of 662 flies of known age is recorded. It becomes evident that the temperature effective period falls between 25 and 35 hours of development under these controlled conditions.

TABLE 4  
*Data on the temperature effective period of mottled and non-mottled as observed by hourly transfers from 27°C. to 18°C.*

| Hours at 27°C | 24         |                | 25         |                | 26         |                | 27         |                | 28         |                | 29         |                |
|---------------|------------|----------------|------------|----------------|------------|----------------|------------|----------------|------------|----------------|------------|----------------|
|               | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> |
| Individuals   | 8          |                | 20         |                | 50         | 3              | 31         | 1              | 32         | 17             | 10         | 26             |
| Hours at 27°C | 30         |                | 31         |                | 32         |                | 33         |                | 34         |                |            |                |
|               | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> |            |                |
| Individuals   | 6          | 37             | 4          | 31             |            | 51             | 1          | 60             | 2          | 71             |            |                |
| Hours at 27°C | 35         |                | 36         |                | 37         |                | 38         |                | 39         |                |            |                |
|               | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> | <i>mot</i> | <i>non-mot</i> |            |                |
| Individuals   | 75         |                | 43         |                | 29         |                | 32         |                | 31         |                |            |                |

The visible expression of the mutant is, however, much later. This time was determined in the following way. Pupae were embedded in modeling clay on the white stage of a binocular microscope. In this way the anterior portion of the pupa case was carefully removed with a pair of very sharp needles, thus exposing the developing eye. It was soon found in dissecting pupae cases of different ages that the deposition of the brown pigmentation preceded the vermilion. This is exemplified in the photo-

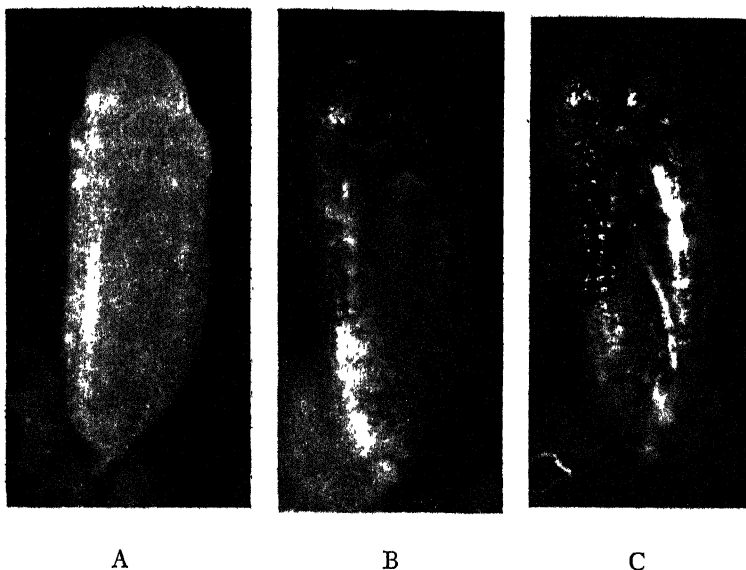


FIGURE 1.—Photomicrographs of dissected pupae showing (A) no pigmentation, (B) dark brown deposition and (C) dark brown deposition and vermilion pigmentation.

micrographs (fig. 1) of such dissected pupae. These photomicrographs were taken through a 4 mm. Bausch and Lomb microscope objective directly

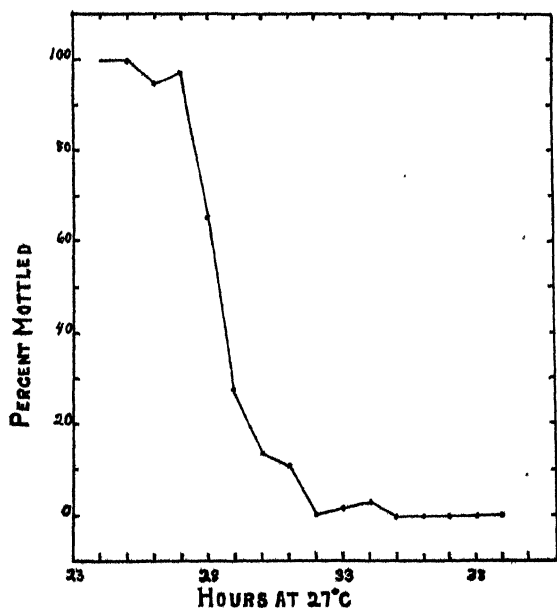


FIGURE 2.—Graphic relation of the temperature effective period.

adapted in place of the lens on a  $3\frac{1}{4} \times 4\frac{1}{4}$  inch plate camera. A shows no eye coloration, B shows the dark pigmentation but no vermilion, and C shows the dark brown pigmentation and the incoming vermilion coloration.

Figure 2 is a graphic representation of the percentage of flies examined which showed the mutation at various hours of exposure to a temperature of  $27^{\circ}\text{C}$ .

It was observed that the dark brown pigmentation on the eye did not spread in extent after it once became visible, but gradually became more sharply differentiated from surrounding tissue.

Large families of flies were again made up, as previously described, for spontaneous pupae production. The cases were removed at hourly intervals and handled as in the previously described fashion. After the pupae were incubated at  $27^{\circ}\text{C}$  for 24 hours they were placed at  $18^{\circ}\text{C}$ . At the end of an additional 48 hours the pupae were carefully embedded in modeling clay, which had previously been molded into suitable shape on a removable Spencer binocular stage plate. The clay and plate were placed in the incubator so that their temperature would be that of the pupae. As the pupae were successfully dissected they were placed on microscope slides in definite positions, which had previously been determined by two rows of numbers. In this way the exact age of the pupae could be easily recorded. When the slide was filled with pupa cases it was slipped on to a carrier which was made of two strips of glass with an air space of about  $\frac{1}{2}$  inch between them. Both the carrier and the numbered slides had been incubated at  $18^{\circ}\text{C}$  so that the pupae would not change temperature greatly when mounted. This was an attempt to have a means of observation at future hourly intervals without significant temperature changes. The slides were slipped off the carrier after each observation and both slides and carrier remained in the incubator until the next observation.

The first group of pupae were taken at six different consecutive hourly intervals. They were dissected and observed at hourly intervals for color deposition at room temperatures ( $22^{\circ}$ – $25^{\circ}\text{C}$ ). The second group of pupae were taken at eight different consecutive hourly intervals. They were dissected and observed at hourly intervals for color deposition in a cold room ( $10^{\circ}$ – $15^{\circ}\text{C}$ , during December).

It became evident in these two experiments that the hourly observations were more frequent than necessary, because hourly changes were not recordable and further it changed environmental conditions unnecessarily. The third group of pupae were all taken at a single hourly interval. They were dissected and observed at three hour intervals in a constant  $18^{\circ}\text{C}$  thermostatically controlled chamber. The data of the three experiments are recorded in table 5.

From these data it becomes evident that temperature is of vital importance in the time of pigment deposition of both brown and vermilion. In the first group of 12 pupae taken at six different consecutive hourly

TABLE 5  
*Recorded data of the time of brown and vermilion deposition in individuals of experiments I, II and III.*

| TIME OF INDIVIDUAL BROWN COLORATION AFTER PUPATION<br>(8 HR. INTERVALS) |      |     | TIME OF VERMILION COLOR APPEARANCE<br>(3 HR. INTERVALS) |       |       |
|---|------|-----|---|-------|-------|
| I   | II   | III | I   | II    | III   |
| 87  | 96   | 91  | 102   | 114   | 109   |
| 87  | 99   | 94  | 105   | 114   | 109   |
| 89  | 100  | 94  | 104   | 127   | 109   |
| 90  | 103  | 94  | 110   | 127   | 109   |
| 90  | 103  | 94  | 108   | 127   | 109   |
| 90  | 103  | 94  | 105   | 127   | 109   |
| 91  | 103  | 94  | 103   | 130   | 109   |
| 91  | 104  | 94  | 100   | 116   | 109   |
| 91  | 104  | 94  | 106   | 122   | 109   |
| 92  | 104  | 94  | 110   | 122   | 109   |
| 92  | 104  | 94  | 110   | 125   | 109   |
| 101   | 104  | 94  | 107   | 125   | 109   |
|   | 104  | 94  |   | 125   | 112   |
|   | 104  | 94  |   | 125   | 112   |
|   | 104  | 94  |   | 125   | 112   |
|   | 104  | 94  |   | 125   | 112   |
|   | 104  | 94  |   | 128   | 112   |
|   | 105  | 94  |   | 120   | 112   |
|   | 105  | 94  |   | 126   | 112   |
|   | 106  | 94  |   | 115   | 112   |
|   | 106  | 97  |   | 127   | 109   |
|   | 106  |     |   | 130   |       |
|   | 108  |     |   | 123   |       |
|   | 108  |     |   | 126   |       |
|   | 108  |     |   | 126   |       |
|   | 108  |     |   | 129   |       |
|   | 108  |     |   | 129   |       |
|   | 112  |     |   | 127   |       |
|   | 112  |     |   | 127   |       |
| Mean  | 90.8 | 94  | 105.8   | 124.1 | 110.9 |

intervals there is a range from 87 to 101 hours in the time of the deposition of brown, or a difference of 14 hours. The vermilion color change varies from 100 to 110 hours after pupation, a difference of 10 hours. The dissection of pupae and interval of observation under a binocular were carried out at 22°-25°C. Thus the cases were at a temperature above 18°C

during some of their development and the rate of change would be expected to be increased. The second group of 29 individuals show a range of from 96 to 112 hours in the time of the brown deposition or a difference of 16 hours. The vermilion color change varies from 114 to 130 hours—a range of 16 hours. The dissection of pupae and of observation were carried out in a cold room  $10^{\circ}$  to  $15^{\circ}\text{C}$ . Thus the temperature of the pupae was below

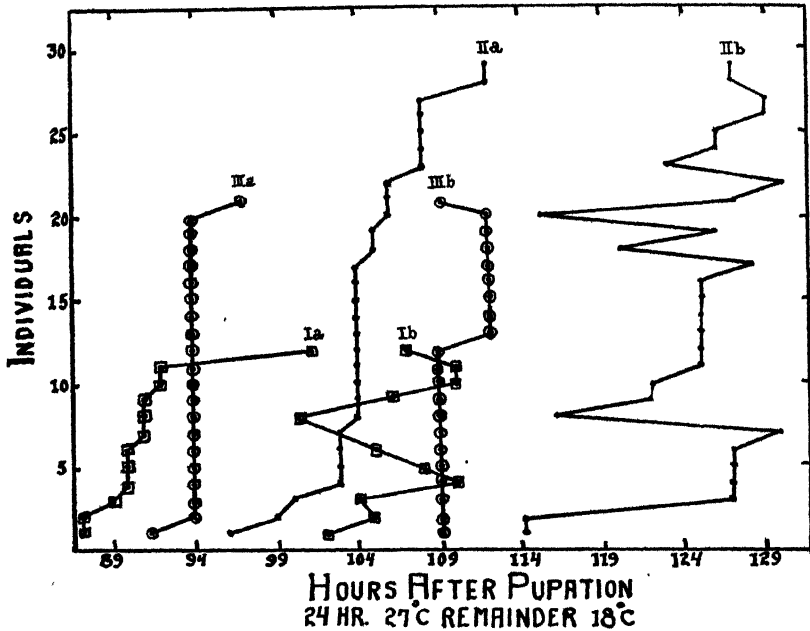


FIGURE 3.—Graphic relation of experiments I, II, and III. Ia, IIa, and IIIa indicate the time at which the various individuals showed brown deposition. Ib, IIb, and IIIb indicate the time the same individuals showed vermilion deposition.

$18^{\circ}\text{C}$  during some of their development and the rate of change would be expected to be decreased.

If the temperatures of observation and dissection were of significance, the third group of pupae dissected and observed at  $18^{\circ}\text{C}$  should fall between the other two. Such is the case as is shown graphically in figure 3. The third group of 21 pupae were all taken at the same hour interval and show a range of 6 hours for brown and 3 for vermilion.

There are, however, other reasons for the variation. In groups I and II the pupae were taken at six and eight different consecutive hourly intervals respectively. Thus on no two occasions would environmental conditions be identical. The degree of injury of an individual at the time of dissection and the time at which it occurred would undoubtedly retard or accelerate certain processes. Nevertheless, it becomes evident that the actual ap-



pearance of the mottling occurs long after the temperature effective period for its development. This is indicated in figure 4, in which the mean of the effective period obtained from 662 individuals falls at 30 hours ( $\pm 5$  hrs.). The mean of actual appearance of brown and vermilion obtained from 21 individuals of the third group is indicated by the horizontal line (24 hours at  $27^{\circ}\text{C}$  and the remainder of development at  $18^{\circ}\text{C}$ ). Thus the mean point

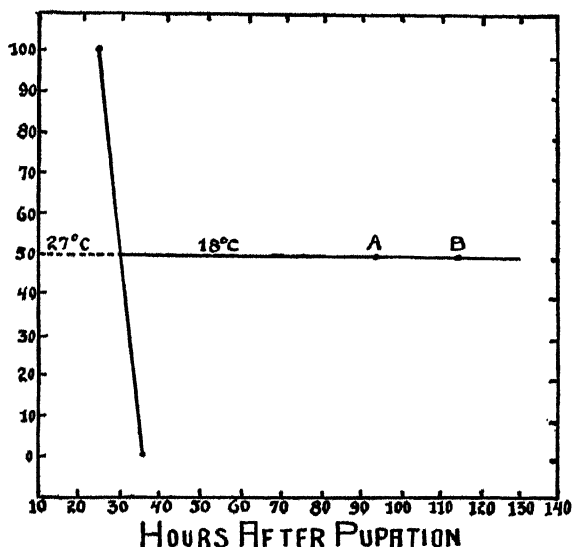


FIGURE 4.—The horizontal line is the graphic relation (A) of the mean time of actual brown deposition; (B) the mean time of the actual vermilion deposition to the temperature effective period.

of brown deposition falls at 94 hours, and that of vermilion at 110.9 hours after pupation. (It should be remembered that observations are recorded at three hour intervals, and the range of brown deposition is from 91-97 hours and vermilion from 109-112.)

#### DISCUSSION

The mottled mutant here described is one of extreme environmental sensitivity. It was shown by the author (1935) that at temperatures of  $20^{\circ}\text{C}$  or below the brown pigmentation was expressed; while at temperatures of  $25^{\circ}\text{C}$  or above it was erased.

The character is sharp in its expression. Neighboring facets are either darkly pigmented or free from the pigment entirely. This undoubtedly means that no dilution occurs. Apparently the molecular size of the pigment is sufficient to be unable to diffuse to neighboring facets. The number of facets effected and thus the size of the area of brown pigmentation dep-

osition is roughly inversely proportional to the temperature (SURRARRER 1935). The difference in the color of the eye is in accord with changes in temperature apparently in the manner of a chemical reaction. Comparable changes as a result of temperature have frequently been recorded with special reference to wing and eye mutants in *Drosophila*.

The temperature effective period falls within the pupae stage and is between 25 and 35 hours after pupation at 27°C. This period is the time limit from the first individual to the last to show the temperature effect.

The actual appearance of the brown pigmentation as would be generally expected falls definitely after the sensitive period. Under the most carefully controlled conditions it appears between 91 and 97 hours; while the vermilion deposition comes considerably later, between 109 and 112 hours. (It should be kept in mind that a three hour observation interval was used.) These data conform with the results of SCHULTZ (1935) which showed that in eye mutants of dark pigmentation the color deposition was definitely ahead of the light or more dilute eye mutants.

If genic action as expressed by mutants is the acceleration or retardation of normal processes, the mottled vermilion combination affords fortunate experimental material. SCHULTZ (1935) showed in *Drosophila* that the sequence of pigment deposition in the eye was yellow to tan to red. This is also confirmed by the work of COCHRANE (1936) in *Drosophila pseudo-obscura*, which shows further that eye color may be the effect produced by two or more genes, each acting in its own sensitive period. Thus the early appearance of the brown deposition (the temperature effective period of which is known) in reference to vermilion (whose temperature effective period is not known) would physically obstruct the appearance of the latter.

Mottling of the eyes in *Drosophila* may be attributed to several types of changes or various combinations of them.

(1) Point mutation or chromosome change, although always a possibility apparently has no substantial proof. MULLER (1930) states that visible variations induced by X-rays which involve point mutations are similar to spontaneous visible variations; and that such simple point mutations are stable in their inheritance. No eversporting cases have been found.

(2) Somatic disjunction or reduction of chromatin provided possible explanation for MORGAN (1911) and SPENCER (1926, 1930).

(3) Somatic crossing over and segregation (STERN 1935).

(4) Inversion or translocation resulting in probable somatic elimination of chromatin (PATTERSON 1928, 1932, 1933; PATTERSON and PAINTER 1931; VAN ATTA 1932; GOWEN and GAY 1934).

(5) Inversion or translocation resulting in a positional change of inert

material from the region of the spindle fiber to other active loci; or perhaps better, the relations of inert material to active loci (GOWEN and GAY 1933; SCHULTZ 1936).

(6) Unstable gene hypothesis (DEMEREK and SLIZYNSKA 1937).

A frequently recorded type is that of X-ray induced translocation with apparent somatic elimination. It seems probable that the mottled here concerned is a somewhat stable nuclear change, highly dependent upon environmental conditions. This seems evident because (1) at 20°C or below the character is expressed in 100 percent of a population; while at temperatures of 25°C or above it is completely absent. (2) The lower the temperature the greater is the area of the eye affected. It seems that the molecular change in the formation of the pigment is only possible at relatively low temperatures. (3) The time of the temperature effective period is sufficiently late in the cycle to remove the possibility of somatic change. (4) Flies whose eyes are non-mottled as a result of the temperature effect, produce mottled offspring if the pupae are held at temperatures of 20°C or below.

Mottled is on the third chromosome and may involve more than a point mutation. It is evident, however, that the region of the spindle fiber is involved. The temperature effective period of the mutant is rather sharply defined as is also the time of its actual expression.

#### SUMMARY

1. Mottled acts as a typical Mendelian recessive and is very sensitive to temperature.
2. Adults with the mottled character erased because of development at 25°C or above produce progeny with mottling expressed if the pupae develop at suitable temperatures.
3. Mottled is on the third chromosome as located by the dominant physiologically balanced stock *S/C'y D/C IIIX*. This is further confirmed by linkage data.
4. By three point linkage data the mottled character was placed at 5.46 crossover units from *Dichaete* and 11.87 units from *Stubble*. It thus involves the region of the spindle fiber attachment.
5. Mottled is a rather stable nuclear change, and not a result of somatic elimination or mutation.
6. The temperature effective period falls between 25 and 35 hours after pupation at 27°C.
7. The expression of mottled occurs 91 to 97 hours after pupation (24 hours at 27°C and the remaining development at 18°C). The vermilion coloration occurs between 109 and 112 hours after pupation (24 hours at 27°C and the remaining development at 18°C).

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